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NOVEL GENES ENCODING PROTEINS HAVING PROGNOSTIC, DIAGNOSTIC, PREVENTIVE, THERAPEUTIC AND OTHER USES

TECHNICAL FIELD OF THE INVENTION

This invention relates to polypeptides and the genes encoding them.

RELATED APPLICATIONS

This Application is a continuation of U.S. Application No. 09/803,589, filed March 9, 2001 which is a continuation-in-part (and claims the benefit of priority under 35 USC 120) of the following applications:

1. U.S. Application Serial No. 09/128,709 (filed August 4, 1998), which application claims priority from U.S. Serial No. 60/054,645 (filed August 4, 1997).

2. U.S. Application Serial No. 09/130,491 (filed August 6, 1998), which application claims priority from U.S. Serial No. 60/054,966 (filed August 6, 1997) and U.S. Serial No. 60/058,108 (filed September 5, 1997).

3. U.S. Application Serial No. 09/388,280 (filed September 1, 1999), a divisional of U.S. Application Serial No. 09/130,491 (filed August 6, 1998), which application claims priority from U.S. Serial No. 60/054,966 (filed August 6, 1997) and U.S. Serial No. 60/058,108 (filed September 5, 1997).

4. U.S. Application Serial No. 09/388,279 (filed September 1, 1999), a divisional of U.S. Application Serial No. 09/130,491 (filed August 6, 1998), which application claims priority from U.S. Serial Number 60/054,966 (filed August 6, 1997) and U.S. Serial Number 60/058,108 (filed September 5, 1997). The entire teachings of U.S. Application Nos: 09/803,589, 09/128,709, 09/130,491, 09/388,280 and 09/388,279 are incorporated herein by reference.

SUMMARY OF THE INVENTION

The invention relates to the discovery and characterization of the genes encoding Tango-71, Tango-79, and Tango-81. Tango-71 (SEQ ID NO:1; FIG. 5) encodes a human protein (SEQ ID NO:2; FIG. 5) that is approximately 90% identical to murine ADAMTS-1 (SEQ ID NO:8; FIG. 6). Tango-79 cDNA (SEQ ID NO:3; FIG. 1) was isolated from a human fetal brain library (Clontech; Palo Alto, CA) and encodes a 615 amino acid protein (SEQ ID NO:4; FIG. 1) that is homologous to *Drosophila Melanogaster* slit protein

(Taguchi et al., *Mol. Brain Res.* 35:31, 1996). Tango-81 cDNA (SEQ ID NO:5; FIG. 2) was isolated from a human fetal brain library and encodes a 261 amino acid protein (SEQ ID NO:6; FIG. 2). The invention also includes murine Tango-71 nucleic acid (SEQ ID NO:9; FIG. 7) and polypeptide (SEQ ID NO:10; FIG. 7), murine Tango-79 nucleic acid (SEQ ID NO:11; FIG. 8) and polypeptide (SEQ ID NO:12), and murine Tango-81 nucleic acid (SEQ ID NO:13; FIG. 9) and polypeptide (SEQ ID NO:14).

The invention features isolated nucleic acid molecules encoding Tango-71, Tango-79, or Tango-81 polypeptides, isolated nucleic acid molecules that encode polypeptides that are substantially identical to the Tango-71, Tango-79, or Tango-81 protein sequences described herein (SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:10, SEQ ID NO:12, or SEQ ID NO:14) and isolated nucleic acid molecules which hybridize under stringent conditions to the protein coding portions of the Tango-71, Tango-79, or Tango-81 nucleic acid sequences described herein (SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:9, SEQ ID NO:11, or SEQ ID NO:13).

The invention also features a host cell that includes an isolated nucleic acid molecule encoding a polypeptide of the invention and a nucleic acid vector (e.g., an expression vector; a vector which includes a regulatory element; a vector that is a virus; a vector that is a retrovirus) containing an isolated nucleic acid molecule encoding a polypeptide of the invention.

In one embodiment, the invention features a substantially pure polypeptide of the invention (e.g., a polypeptide of the invention that is soluble under physiological conditions); a polypeptide of the invention that includes a signal sequence; a Tango-71 polypeptide that is at least 85%, 90%, 95%, or 100% identical to the amino acid sequence of SEQ ID NO:2 or SEQ ID NO:10; a Tango-79 polypeptide that is at least 85%, 90%, 95%, or 100% identical to the amino acid sequence of SEQ ID NO:4 or SEQ ID NO:12; a Tango-81 polypeptide that is at least 85%, 90%, 95%, or 100% identical to the amino acid sequence of SEQ ID NO:6 or SEQ ID NO:14.

In other embodiments the invention also features substantially pure polypeptides which include a first portion and a second portion, the first portion including a polypeptide of the invention and the second portion including a detectable marker.

The invention also features antibodies, e.g., monoclonal, polyclonal, and engineered antibodies, which specifically bind polypeptides of the invention. By "specifically binds" is meant an antibody that recognizes and binds to a particular antigen, e.g., a Tango-71, Tango-79,

or Tango-81 polypeptide of the invention, but which does not substantially recognize or bind to other molecules in a sample, e.g., a biological sample, which includes the polypeptide (e.g., Tango-71, Tango-79, or Tango-81).

The invention also features a pharmaceutical composition that includes a polypeptide of the invention.

The invention includes methods for diagnosing a disorder associated with aberrant expression of a protein of the invention (i.e., Tango-71, Tango-79, or Tango-81), the method including obtaining a biological sample from a patient and measuring the expression of the protein in the biological sample, wherein increased or decreased expression of the protein in the biological sample compared to a control indicates that the patient suffers from a disorder associated with aberrant expression of the protein.

The invention encompasses isolated nucleic acid molecules encoding a polypeptide of the invention or a polypeptide fragment thereof; vectors containing these nucleic acid molecules; cells harboring recombinant DNA encoding a polypeptide of the invention; fusion proteins which include all or a portion of a polypeptide of the invention; transgenic animals which express a polypeptide of the invention; and recombinant knock-out animals which fail to express a polypeptide of the invention.

The nucleic acid molecules of the invention can be inserted into vectors, as described below, which will facilitate expression of the insert. The nucleic acid molecules and the polypeptides they encode can be used directly as diagnostic or therapeutic agents, or (in the case of a polypeptide) can be used to generate antibodies that, in turn, are therapeutically useful. Accordingly, expression vectors containing the nucleic acid molecules of the invention, cells transfected with these vectors, the polypeptides expressed, and antibodies generated (against either the entire polypeptide or an antigenic fragment thereof) are among the preferred embodiments.

A transformed cell is any cell into which (or into an ancestor of which) has been introduced, by means of recombinant DNA techniques, a nucleic acid encoding a polypeptide of the invention (e.g., a Tango-71, Tango-79, or Tango-81 polypeptide).

The invention also encompasses nucleic acid molecules that hybridize, preferably under stringent conditions, to a nucleic acid molecule encoding a polypeptide of the invention (e.g., the polypeptide encoding portions of a nucleic acid molecule having the sequence shown in SEQ ID

3

NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:9, SEQ ID NO:11, or SEQ ID NO:13). Preferably the hybridizing nucleic acid molecule consists of 400, more preferably 200 nucleotides. Preferred hybridizing nucleic acid molecules have a biological activity possessed by a nucleic acid of the invention.

The invention also features substantially pure or isolated polypeptides of the invention, including those that correspond to various functional domains of polypeptides of the invention, or fragments thereof.

The polypeptides of the invention can be prepared by recombinant gene expression, chemically synthesized, or purified from tissues in which they are naturally expressed using standard biochemical methods of purification.

Also included in the invention are functional polypeptides, which possess one or more of the biological functions or activities of Tango-71, Tango-79, or Tango-81. These functions include the ability to bind some or all of the proteins that normally bind to polypeptides of the invention. A functional polypeptide is also considered within the scope of the invention if it serves as an antigen for production of antibodies that specifically bind to a polypeptide of the invention. In many cases, functional polypeptides retain one or more domains present in the naturally-occurring form of the polypeptide.

The functional polypeptides may contain a primary amino acid sequence that has been modified from those disclosed herein. Preferably these modifications consist of conservative amino acid substitutions, as described herein.

Another aspect of this invention features isolated or recombinant proteins and polypeptides of the invention, or modulators thereof. Preferred proteins and polypeptides possess at least one biological activity possessed by the corresponding naturally-occurring human polypeptide. An activity, a biological activity, and a functional activity of a polypeptide of the invention refers to an activity exerted by a protein or polypeptide of the invention on a responsive cell as determined *in vivo*, or *in vitro*, according to standard techniques. Such activities can be a direct activity, such as an association with or an enzymatic activity on a second protein or an indirect activity, such as a cellular signaling activity mediated by interaction of the protein with a second protein. Thus, such activities include, *e.g.*, (1) the ability to form protein-protein interactions with proteins in the signaling pathway of the naturally-occurring

4

polypeptide; (2) the ability to bind a ligand of the naturally-occurring polypeptide; (3) the ability to bind to an intracellular target of the naturally-occurring polypeptide.

Further activities of polypeptides of the invention include the ability to modulate (this term, as used herein, includes, but is not limited to, stabilize, promote. inhibit or disrupt, protein-protein interactions (e.g., homophilic and/or heterophilic)), protein-ligand interactions, e.g., in receptor-ligand recognition, development, differentiation, maturation, proliferation and/or activity of cells function, survival, morphology, proliferation and/or differentiation of cells of tissues in which it is expressed. Additional activities include but are not limited to: (1) the ability to modulate cell surface recognition; (2) the ability to transduce an extracellular signal (e.g., by interacting with a ligand and/or a cell-surface receptor); (3) the ability to modulate a signal transduction pathway; and (4) the ability to modulate intracellular signaling cascades (e.g.. signal transduction cascades).

The invention also features antagonists and agonists of Tango-71, Tango-79, or Tango-81 that can inhibit or enhance, respectively, one or more of the biological activities of nucleic acids or polypeptides of the invention. Suitable antagonists can include: small molecules (i.e., molecules with a molecular weight below about 500); large molecules (i.e., molecules with a molecular weight above about 500); antibodies that bind and "neutralize" polypeptides of the invention (as described below); polypeptides that compete with a native form of a polypeptide of the invention for binding to a functional binding partner of the native protein of the invention; and nucleic acid molecules that interfere with transcription of nucleic acids of the invention (for example, antisense nucleic acid molecules and ribozymes). Agonists of nucleic acids or polypeptides of the invention also include small and large molecules, and antibodies other than neutralizing antibodies.

In addition, the invention features substantially pure polypeptides that functionally interact with polypeptides of the invention and the nucleic acid molecules that encode them.

The invention encompasses methods for treating disorders associated with aberrant expression or activity of a protein of the invention (i.e., Tango-71, Tango-79, or Tango-81). Thus, the invention includes methods for treating disorders associated with excessive expression or activity of a protein of the invention. Such methods entail administering a compound that decreases the expression or activity of the protein. The invention also includes methods for treating disorders associated with insufficient expression or activity of a protein of the invention.

These methods entail administering a compound that increases the expression or activity of the protein.

The invention also features methods for detecting a protein of the invention (i.e., Tango-71, Tango-79, or Tango-81). Such methods include: obtaining a biological sample; contacting the sample with an antibody that specifically binds to the protein under conditions that permit specific binding; and detecting any antibody-protein complexes formed.

In addition, the present invention encompasses methods and compositions for the diagnostic evaluation, typing, and prognosis of disorders associated with inappropriate expression or activity of nucleic acids or polypeptides of the invention. For example, the nucleic acid molecules of the invention can be used as diagnostic hybridization probes to detect, for example, inappropriate expression of nucleic acids or polypeptides of the invention or mutations in the genes of the invention. Such methods may be used to classify cells by the level of expression of nucleic acids or polypeptides of the invention.

Thus, the invention features a method for diagnosing a disorder associated with aberrant activity of a protein of the invention, the method including obtaining a biological sample from a patient and measuring the activity of the protein in the biological sample, wherein increased or decreased activity in the biological sample compared to a control indicates that the patient suffers from a disorder associated with aberrant activity of the protein.

The nucleic acid molecules of the invention can be used as primers for diagnostic PCR analysis for the identification of gene mutations, allelic variations, and regulatory defects in a gene of the invention. The present invention further provides for diagnostic kits for the practice of such methods.

The invention features methods of identifying compounds that modulate the expression or activity of a protein of the invention by assessing the expression or activity of the protein in the presence and absence of a selected compound. A difference in the level of expression or activity of the protein in the presence and absence of the selected compound indicates that the selected compound is capable of modulating expression or activity of the protein. Expression can be assessed either at the level of gene expression (e.g., by measuring mRNA) or protein expression by techniques that are well known to skilled artisans. The activity of nucleic acids or polypeptides of the invention can be assessed functionally.

The preferred methods and materials are described below in examples that are meant to illustrate, not limit, the invention. Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, the preferred methods and materials are described herein. All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety. In the case of conflict, the present specification, including definitions, will control. In addition, the materials, methods, and examples are illustrative only and are not intended to be limiting.

The details of one or more embodiments of the invention are set forth in the accompanying drawings and the description below. Other features, objects, and advantages of the invention will be apparent from the description and drawings, and from the claims.

DESCRIPTION OF DRAWINGS

Figure 1 depicts the nucleic acid sequence (SEQ ID NO:3) and deduced amino acid sequence (SEQ ID NO:4) of human Tango-79. The open reading frame extends from nucleotide 131 to1975.

Figure 2 depicts the nucleic acid sequence (SEQ ID NO:5) and deduced amino acid sequence (SEQ ID NO:6) of human Tango-81. The open reading frame extends from nucleotide 58 to 840.

Figure 3 depicts an alignment between the amino acid sequence of human Tango-79 (SEQ ID NO:3) and D45913 (Leucine rich repeat protein) (SEQ ID NO:7). The sequences show 29.412% identity.

Figure 4 depicts the results of Northern blot analysis of Tango-81 expression.

Figure 5 depicts the nucleic acid sequence (SEQ ID NO:1) and deduced amino acid sequence (SEQ ID NO:2) of human Tango-71. The open reading frame extends from nucleotide 3 to 1829.

Figure 6 depicts an alignment between the amino acid sequence of human Tango-71 (SEQ ID NO:2) and the amino acid sequence of ADAMTS-1 (SEQ ID NO:8). The sequences show 90% identity.

Figure 7 depicts the nucleic acid sequence (SEQ ID NO:9) and deduced amino acid sequence (SEQ ID NO:10) of murine Tango-71. The open reading frame extends from nucleotide 9 to 1562.

Figure 8 depicts the nucleic acid sequence (SEQ ID NO:11) and deduced amino acid sequence (SEQ ID NO:12) of murine Tango-79. The open reading frame extends from nucleotide 323 to 1108.

Figure 9 depicts the nucleic acid sequence (SEQ ID NO:13) and deduced amino acid sequence (SEQ ID NO:14) of murine Tango-81. The open reading frame extends from nucleotide 106 to 630.

DETAILED DESCRIPTION OF THE INVENTION

The present invention is based, at least in part, on the discovery of a variety of cDNA molecules which encode proteins that are herein designated Tango-71, Tango-79, and Tango-81. These proteins exhibit a variety of physiological activities, and are included in a single application for the sake of convenience. It is understood that the allowability or non-allowability of claims directed to one of these proteins has no bearing on the allowability of claims directed to the others. The characteristics of each of these proteins and the cDNAs encoding them are described separately in the ensuing sections. In addition to the full length mature and immature human proteins described in the following sections, the invention includes fragments, derivatives, and variants of these proteins, as described herein. These proteins, fragments, derivatives, and variants are collectively referred to herein as polypeptides of the invention or proteins of the invention.

An "isolated nucleic acid molecule" is a nucleic acid molecule that is separated from the 5' and 3' coding sequences with which it is immediately contiguous in the naturally occurring genome of an organism. Isolated nucleic acid molecules include nucleic acid molecules that are not naturally occurring, e.g., nucleic acid molecules created by recombinant DNA techniques. Nucleic acid molecules include both RNA and DNA, including cDNA, genomic DNA, and synthetic (e.g., chemically synthesized) DNA. Where single-stranded, the nucleic acid molecule may be a sense strand or an antisense strand.

As used herein, a "signal sequence" includes a peptide of at least about 15 or 20 amino acid residues in length which occurs at the N-terminus of secretory and membrane-bound proteins and which contains at least about 70% hydrophobic amino acid residues such as alanine, leucine, isoleucine, phenylalanine, proline, tyrosine, tryptophan, or valine. In a preferred embodiment, a signal sequence contains at least about 10 to 40 amino acid residues, preferably about 19-34 amino acid residues, and has at least about 60-80%, more preferably at least about

8

aspartic acid and glutamic acid; asparagine and glutamine; serine and threonine; lysine and arginine; and phenylalanine and tyrosine.

To determine the percent identity of two amino acid sequences or of two nucleic acids, the sequences are aligned for optimal comparison purposes (e.g., gaps can be introduced in the sequence of a first amino acid or nucleic acid sequence for optimal alignment with a second amino or nucleic acid sequence). The amino acid residues or nucleotides at corresponding amino acid positions or nucleotide positions are then compared. When a position in the first sequence is occupied by the same amino acid residue or nucleotide as the corresponding position in the second sequence, then the molecules are identical at that position. The percent identity between the two sequences is a function of the number of identical positions shared by the sequences (i.e., % identity = # of identical positions/total # of positions (e.g., overlapping positions) x 100). Preferably, the two sequences are the same length.

The determination of percent homology between two sequences can be accomplished using a mathematical algorithm. A preferred, non-limiting example of a mathematical algorithm utilized for the comparison of two sequences is the algorithm of Karlin and Altschul (1990) *Proc. Natl. Acad. Sci. USA* 87:2264-2268, modified as in Karlin and Altschul (1993) *Proc. Natl. Acad. Sci. USA* 90:5873-5877. Such an algorithm is incorporated into the NBLAST and XBLAST programs of Altschul, et al. (1990) *J. Mol. Biol.* 215:403-410. BLAST nucleotide searches can be performed with the NBLAST program, score = 100, wordlength = 12 to obtain nucleotide sequences homologous to Tango-71, Tango-79, or Tango-81 nucleic acid molecules of the invention. BLAST protein searches can be performed with the XBLAST program, score = 50, wordlength = 3 to obtain amino acid sequences homologous to Tango-71, Tango-79, or Tango-81 protein molecules of the invention. To obtain gapped alignments for comparison purposes, Gapped BLAST can be utilized as described in Altschul et al. (1997) *Nucleic Acids Res.* 25:3389-3402. Alternatively, PSI-Blast can be used to perform an iterated search that detects distant relationships between molecules. Id. When utilizing BLAST, Gapped BLAST, and PSI-Blast programs, the default parameters of the respective programs (e.g., XBLAST and NBLAST) can be used. *See* http://www.ncbi.nlm.nih.gov. Another preferred, non-limiting example of a mathematical algorithm utilized for the comparison of sequences is the algorithm of Myers and Miller, (1988) *CABIOS* 4:11-17. Such an algorithm is incorporated into the ALIGN program (version 2.0) which is part of the GCG sequence alignment software package. When

10

utilizing the ALIGN program for comparing amino acid sequences, a PAM120 weight residue table, a gap length penalty of 12, and a gap penalty of 4 can be used.

Another preferred, non-limiting example of a mathematical algorithm utilized for the comparison of sequences is the local homology algorithm of Smith and Waterman (Advances in Applied Mathematics 2: 482-489 (1981)). Such an algorithm is incorporated into the BestFit program, which is part of the Wisconsin™ package, and is used to find the best segment of similarity between two sequences. BestFit reads a scoring matrix that contains values for every possible GCG symbol match. The program uses these values to construct a path matrix that represents the entire surface of comparison with a score at every position for the best possible alignment to that point. The quality score for the best alignment to any point is equal to the sum of the scoring matrix values of the matches in that alignment, less the gap creation penalty multiplied by the number of gaps in that alignment, less the gap extension penalty multiplied by the total length of all gaps in that alignment. The gap creation and gap extension penalties are set by the user. If the best path to any point has a negative value, a zero is put in that position.

After the path matrix is complete, the highest value on the surface of comparison represents the end of the best region of similarity between the sequences. The best path from this highest value backwards to the point where the values revert to zero is the alignment shown by BestFit. This alignment is the best segment of similarity between the two sequences. Further documentation can be found at http://ir.ucdavis.edu/GCGhelp/bestfit.html#algorithm.

Additional algorithms for sequence analysis are known in the art and include ADVANCE and ADAM as described in Torellis and Robotti (1994) Comput. Appl. Biosci., 10:3-5; and FASTA described in Pearson and Lipman (1988) Proc. Natl. Acad. Sci. 85:2444-8. Within FASTA, ktup is a control option that sets the sensitivity and speed of the search. If ktup=2, similar regions in the two sequences being compared are found by looking at pairs of aligned residues; if ktup=1, single aligned amino acids are examined. ktup can be set to 2 or 1 for protein sequences, or from 1 to 6 for DNA sequences. The default if ktup is not specified is 2 for proteins and 6 for DNA. For a further description of FASTA parameters, see http://bioweb.pasteur.fr/docs/man/man/fasta.1.html#sect2, the contents of which are incorporated herein by reference.

The percent identity between two sequences can be determined using techniques similar to those described above, with or without allowing gaps. In calculating percent identity, typically exact matches are counted.

As used herein, the phrase "allelic variant" refers to a nucleotide sequence that occurs at a given locus or to a polypeptide encoded by the nucleotide sequence. Allelic variants of any of these genes can be identified by sequencing the corresponding chromosomal portion at the indication location in multiple individuals.

TANGO 71

Tango-71 cDNA (Fig. 5; SEQ ID NO:1) was isolated from human melanocytes as follows: Human melanocytes (Clonetics Corporation; San Diego, CA) were expanded in culture with Melanocyte Growth Media (MGM; Clonetics) according to the recommendations of the supplier. When the cells reached ~80-90% confluence, they were starved in MGM without growth factors for 46 hours. The starved cells were then stimulated with complete MGM supplemented with 20 ng/ml TNF (Gibco BRL; Gaithersburg, MD) and cycloheximide (CHI;40 micrograms/ml) for 4 hours. Total RNA was isolated using the RNeasy Midi Kit (Qiagen; Chatsworth, CA), and the poly A+ fraction was further purified using Oligotex beads (Qiagen).

Three micrograms of poly A+ RNA were used to synthesize a cDNA library using the Superscript cDNA Synthesis kit (Gibco BRL). Complementary DNA was directionally cloned into the expression plasmid pMET7 using the SalI and NotI sites in the polylinker to construct a plasmid library. Transformants were picked and grown up for single-pass sequencing. Additionally, astrocyte cDNA was ligated into the SalI/NotI sites of the ZipLox vector (Gibco BRL) for construction of a lambda phage cDNA library.

The human TANGO 71 cDNA is 3147 base pairs in length (SEQ ID NO:1), and has an open reading frame from nucleotides 3 to 1829 (1827 base pairs) which encodes a 609 residue protein (SEQ ID NO:2)(shown in Figure 5). The mouse TANGO 71 cDNA is 3145 base pairs in length (SEQ ID NO:9), and has an open reading frame from nucleotides 9 to 1562 (1554 base pairs) which encodes a 518 residue protein (SEQ ID NO:10)(shown in Figure 7). The human and mouse TANGO 71 protein sequences are 89.0% identical and 92.1% similar, as determined by an alignment made using ALIGN software (Myers and Miller (1989) CABIOS, ver. 2.0), with a BLOSUM62 scoring matrix, gap opening penalty 12, gap extension penalty 4, and frameshift gap penalty 5.

Northern blot analysis of Tango-71 expression was performed using Tango-71 labeled with ^{32}P-dCTP using the Prime-It kit (Stratagene, LaJolla, CA). Human mRNA blots (MTNI and MTNII; Clontech; Palo Alto, CA) were probed and washed at high stringency as recommended by the manufacturer. Tango-71 is expressed as an approximately 6.0 kb transcript in all tissues: heart brain, placenta, lung, liver, skeletal muscle, kidney, pancreas, spleen, thymus, prostate, testes, ovary, small intestine, colon, PBLs.

In situ hybridization analysis revealed Tango-71 expression in the following tissues: brain (signal observed in the dentate gyrus and the choroid plexus of the lateral and 4th ventricles); eye and harderian gland (signal observed in retina, possibly the ganglion layer); liver (signal seen lining the large vessels or hepatic ducts); kidney (ubiquitous signal); and placenta (ubiquitous signal observed in the labyrinth zone).

The amino acid sequence of a portion of Tango-71 is 90% identical to the amino acid sequence of murine ADAMTS-1 (FIG. 6), a cellular disintegrin and metalloprotease that is thought to be involved in inflammation and development of cancer cachexia (Kuno et al., *J. Biol. Chem.* 272:556, 1997). Based on sequence comparison to ADAMTS-1, Tango-71, using the amino acid numbering in Figure 6, has the following domains: amino acids 1-160 (metalloproteinase domain, partial); amino acids 170-242 (disintegrin domain); amino acids 257-307 (thrombospondin domain). A less apparent thrombospondin domain is present at amino acid 558-608. Portions of Tango-71 shown in Figure 5, but not in Figure 6, may also be homologous ADAMTS-1. Tango-71 may represent the human homolog of ADAMTS-1 or a splice variant thereof.

Tango-71 expression may be androgen regulated. Tango-71 expression in LNCaP cells, an androgen-dependent prostate cancer cell line, is induced by R1881, a testosterone analog. Tango-71 expression is downregulated in LNCaP cells treated with casodex, an anti-androgen.

TANGO 79

Tango-79 cDNA (SEQ ID NO:3; FIG. 1) was isolated from a human fetal brain library (Clontech; Palo Alto, CA). This Tango-79 cDNA encodes a 615 amino acid protein (SEQ ID NO:4; FIG. 1) that is homologous to *Drosophila Melanogaster* slit protein (Taguchi et al., *Mol. Brain Res.* 35:31, 1996). Slit protein belongs to the leucine-rich repeat (LRR) protein family, whose members act as cell adhesion molecules that play crucial roles in *Drosophila* neuronal development (Taguchi et al., *Mol. Brain Res.* 35:31, 1996).

The Tango-79 cDNA (SEQ ID NO:3; FIG. 1) described herein was isolated using the method described in U.S. Serial No. 08/752,307 (filed November 19, 1996), hereby

incorporated by reference. Tango-79 protein (SEQ ID NO:4; FIG. 1) is homologous to D45913 (leucine rich repeat protein) (SEQ ID NO:7; FIG. 3).

The human TANGO 79 cDNA is 2351 base pairs in length (SEQ ID NO:3), and has an open reading frame from nucleotides 131 to 1975 (1845 base pairs) which encodes a 615 residue protein (SEQ ID NO:4)(shown in Figure 1). The mouse TANGO 79 cDNA is 1110 base pairs in length (SEQ ID NO:11), and has an open reading frame from nucleotides 323 to 1108 (786 base pairs) which encodes a 262 residue protein (SEQ ID NO:12)(shown in Figure 8). The human and mouse TANGO 79 protein sequences are 96.5% identical and 96.5% similar, as determined by an alignment made using ALIGN software (Myers and Miller (1989) CABIOS, ver. 2.0), with a BLOSUM62 scoring matrix, gap opening penalty 12, gap extension penalty 4, and frameshift gap penalty 5.

Northern blot analysis of Tango-79 mRNA showed that an approximate 3.0 kB and an approximate 3.5 kB transcript are expressed in the brain. Tango-79 function can be studied by overexpressing the protein in mouse brain.

In situ hybridization analysis revealed Tango-79 expression in the following tissues: brain (very strong signal throughout the cortex); spinal cord (moderate signal in the grey matter); eye and harderian gland (moderate signal in the ganglion and the photoreceptor layer); spleen (weak, ubiquitous signal).

In addition, a secretion assay performed for Tango-79 revealed a 148 kD protein.

TANGO 81

Tango-81 cDNA was isolated from a human fetal brain library using the method described in U.S. Serial No. 08/752,307 (filed November 19, 1996), hereby incorporated by reference.

The human TANGO 81 cDNA is 979 base pairs in length (SEQ ID NO:5), and has an open reading frame from nucleotides 58 to 840 (783 base pairs) which encodes a 261 residue protein (SEQ ID NO:6)(shown in Figure 2). The mouse TANGO 81 cDNA is 1027 base pairs in length (SEQ ID NO:13), and has an open reading frame from nucleotides 106 to 630 (525 base pairs) which encodes a 175 residue protein (SEQ ID NO:14)(shown in Figure 9). The human and mouse TANGO 81 protein sequences are 73.7% identical and 75.4% similar, as determined by an alignment made using ALIGN software (Myers and Miller (1989) CABIOS, ver. 2.0), with a BLOSUM62 scoring matrix, gap opening penalty 12, gap extension penalty 4, and frameshift gap penalty 5.

Northern analysis of Tango-81 expression reveals that it is expressed in heart, brain, spleen, lung, liver, skeletal muscle, kidneys and testis (FIG. 4).

Tango-71, Tango-79, and Tango-81 Nucleic Acid Molecules

The invention encompasses nucleic acids that have a sequence that is substantially identical to the nucleic acid sequence of Tango-71, Tango-79, or Tango-81. A nucleic acid sequence which is substantially identical to a given reference nucleic acid sequence is hereby defined as a nucleic acid having a sequence that has at least 85%, preferably 90%, and more preferably 95%, 98%, 99% or more identity to the sequence of the given reference nucleic acid sequence, e.g., the nucleic acid sequence of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:9, SEQ ID NO:11, or SEQ ID NO:13.

The Tango-71, Tango-79, or Tango-81 nucleic acid molecules of the invention can be cDNA, genomic DNA, synthetic DNA, or RNA, and can be double-stranded or single-stranded (*i.e.*, either a sense or an antisense strand). Fragments of these molecules are also considered within the scope of the invention, and can be produced, for example, by the polymerase chain reaction (PCR) or generated by treatment with one or more restriction endonucleases. A ribonucleic acid (RNA) molecule can be produced by *in vitro* transcription.

The nucleic acid molecules of the invention can contain naturally occurring sequences, or sequences that differ from those that occur naturally, but, due to the degeneracy of the genetic code, encode the same polypeptide. In addition, these nucleic acid molecules are not limited to sequences that only encode polypeptides, and thus, can include some or all of the non-coding sequences that lie upstream or downstream from a coding sequence.

The nucleic acid molecules of the invention can be synthesized (for example, by phosphoramidite-based synthesis) or obtained from a biological cell, such as the cell of a mammal. Thus, the nucleic acids can be those of a human, mouse, rat, guinea pig, cow, sheep, horse, pig, rabbit, monkey, dog, or cat. Combinations or modifications of the nucleotides within these types of nucleic acids are also encompassed.

In addition, the isolated nucleic acid molecules of the invention encompass fragments that are not found as such in the natural state. Thus, the invention encompasses recombinant molecules, such as those in which a nucleic acid molecule (for example, an isolated nucleic acid molecule encoding Tango-71, Tango-79, or Tango-81) is incorporated into a vector (for example, a plasmid or viral vector) or into the genome of a heterologous cell (or the genome of a homologous cell, at a position other than the natural chromosomal location). Recombinant nucleic acid molecules and uses therefor are discussed further below.

In the event the nucleic acid molecules of the invention encode or act as antisense molecules, they can be used for example, to regulate translation of mRNA of the invention. Techniques associated with detection or regulation of expression of nucleic acids or polypeptides of the invention are well known to skilled artisans and can be used to diagnose and/or treat disorders associated with aberrant expression of nucleic acids or polypeptides of the invention.

The invention also encompasses nucleic acid molecules that hybridize under stringent conditions to a nucleic acid molecule encoding a polypeptide of the invention (e.g., nucleic acid molecules having the sequence of the protein encoding portion of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:9, SEQ ID NO:11, or SEQ ID NO:13). The cDNA sequences described herein can be used to identify these hybridizing nucleic acids, which include, for example, nucleic acids that encode homologous polypeptides in other species, and splice variants of the genes of the invention in humans or other mammals. Accordingly, the invention features methods of detecting and isolating these nucleic acid molecules. Using these methods, a sample (for example, a nucleic acid library, such as a cDNA or genomic library) is contacted (or "screened") with a probe specific to a nucleotide of the invention (for example, a fragment of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:9, SEQ ID NO:11, or SEQ ID NO:13 that is at least 25 or 50 or 100 nucleotides long). The probe will selectively hybridize to nucleic acids encoding related polypeptides (or to complementary sequences thereof). The probe, which can contain at least 25 (for example, 25, 50, 100, or 200 nucleotides) can be produced using any of several standard methods (see, for example, Ausubel et al.,"Current Protocols in Molecular Biology, Vol. I," Green Publishing Associates, Inc., and John Wiley & Sons, Inc., NY, 1989). For example, the probe can be generated using PCR amplification methods in which oligonucleotide primers are used to amplify a nucleic acid sequence specific to a nucleic acid of the invention that can be used as a probe to screen a nucleic acid library and thereby detect nucleic acid molecules (within the library) that hybridize to the probe.

One single-stranded nucleic acid is said to hybridize to another if a duplex forms between them. This occurs when one nucleic acid contains a sequence that is the reverse and complement of the other (this same arrangement gives rise to the natural interaction between the sense and antisense strands of DNA in the genome and underlies the configuration of the "double helix"). Complete complementarity between the hybridizing regions is not required in order for a duplex

to form; it is only necessary that the number of paired bases is sufficient to maintain the duplex under the hybridization conditions used.

Typically, hybridization conditions are of low to moderate stringency. These conditions favor specific interactions between completely complementary sequences, but allow some non-specific interaction between less than perfectly matched sequences to occur as well. After hybridization, the nucleic acids can be "washed" under moderate or high conditions of stringency to dissociate duplexes that are bound together by some non-specific interaction (the nucleic acids that form these duplexes are thus not completely complementary).

As is known in the art, the optimal conditions for washing are determined empirically, often by gradually increasing the stringency. The parameters that can be changed to affect stringency include, primarily, temperature and salt concentration. In general, the lower the salt concentration and the higher the temperature, the higher the stringency. Washing can be initiated at a low temperature (for example, room temperature) using a solution containing a salt concentration that is equivalent to or lower than that of the hybridization solution. Subsequent washing can be carried out using progressively warmer solutions having the same salt concentration. As alternatives, the salt concentration can be lowered and the temperature maintained in the washing step, or the salt concentration can be lowered and the temperature increased. Additional parameters can also be altered. For example, use of a destabilizing agent, such as formamide, alters the stringency conditions.

In reactions where nucleic acids are hybridized, the conditions used to achieve a given level of stringency will vary. There is not one set of conditions, for example, that will allow duplexes to form between all nucleic acids that are 85% identical to one another; hybridization also depends on unique features of each nucleic acid. The length of the sequence, the composition of the sequence (for example, the content of purine-like nucleotides versus the content of pyrimidine-like nucleotides) and the type of nucleic acid (for example, DNA or RNA) affect hybridization. An additional consideration is whether one of the nucleic acids is immobilized (for example, on a filter).

An example of a progression from lower to higher stringency conditions is the following, where the salt content is given as the relative abundance of SSC (a salt solution containing sodium chloride and sodium citrate; 2X SSC is 10-fold more concentrated than 0.2X SSC). Nucleic acids are hybridized at 42°C in 2X SSC/0.1% SDS (sodium dodecylsulfate; a detergent)

and then washed in 0.2X SSC/0.1% SDS at room temperature (for conditions of low stringency): 0.2X SSC/0.1% SDS at 42°C (for conditions of moderate stringency); and 0.1X SSC at 68°C (for conditions of high stringency). Washing can be carried out using only one of the conditions given, or each of the conditions can be used (for example, washing for 10-15 minutes each in the order listed above). Any or all of the washes can be repeated. As mentioned above, optimal conditions will vary and can be determined empirically.

A second set of conditions that are considered "stringent conditions" are those in which hybridization is carried out at 50°C in Church buffer (7% SDS, 0.5% NaHPO$_4$, 1 M EDTA, 1% BSA) and washing is carried out at 50°C in 2X SSC.

Once detected, the nucleic acid molecules can be isolated by any of a number of standard techniques (see, for example, Sambrook et al., "Molecular Cloning, A Laboratory Manual," 2nd Ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989).

The invention also encompasses: (a) expression vectors that contain any of the foregoing coding sequences (related to a polypeptide of the invention) and/or their complements (that is, "antisense" sequence); (b) expression vectors that contain any of the foregoing coding sequences (related to a polypeptide of the invention) operatively associated with a regulatory element (examples of which are given below) that directs the expression of the coding sequences; (c) expression vectors containing, in addition to sequences encoding a polypeptide of the invention, nucleic acid sequences that are unrelated to nucleic acid sequences encoding a polypeptide of the invention, such as molecules encoding a reporter or marker; and (d) genetically engineered host cells that contain any of the foregoing expression vectors and thereby express the nucleic acid molecules of the invention in the host cell.

Recombinant nucleic acid molecules can contain a sequence encoding a soluble polypeptide of the invention; mature polypeptide of the invention; or polypeptide of the invention having an added or endogenous signal sequence. A full length polypeptide of the invention; a domain of a polypeptide of the invention; or a fragment thereof may be fused to additional polypeptides, as described below. Similarly, the nucleic acid molecules of the invention can encode the mature form of a polypeptide of the invention or a form that encodes a polypeptide that facilitates secretion. In the latter instance, the polypeptide is typically referred to as a proprotein, which can be converted into an active form by removal of the signal sequence.

18

for example, within the host cell. Proproteins can be converted into the active form of the protein by removal of the inactivating sequence.

The regulatory elements referred to above include, but are not limited to, inducible and non-inducible promoters, enhancers, operators and other elements, which are known to those skilled in the art, and which drive or otherwise regulate gene expression. Such regulatory elements include but are not limited to the cytomegalovirus hCMV immediate early gene, the early or late promoters of SV40 adenovirus, the lac system, the trp system, the TAC system, the TRC system, the major operator and promoter regions of phage A, the control regions of fd coat protein, the promoter for 3-phosphoglycerate kinase, the promoters of acid phosphatase, and the promoters of the yeast α-mating factors.

Similarly, the nucleic acid can form part of a hybrid gene encoding additional polypeptide sequences, for example, sequences that function as a marker or reporter. Examples of marker or reporter genes include β-lactamase, chloramphenicol acetyltransferase (CAT), adenosine deaminase (ADA), aminoglycoside phosphotransferase (neo^r, G418^r), dihydrofolate reductase (DHFR), hygromycin-B-phosphotransferase (HPH), thymidine kinase (TK), lacZ (encoding β-galactosidase), and xanthine guanine phosphoribosyltransferase (XGPRT). As with many of the standard procedures associated with the practice of the invention, skilled artisans will be aware of additional useful reagents, for example, of additional sequences that can serve the function of a marker or reporter. Generally, the hybrid polypeptide will include a first portion and a second portion; the first portion being a polypeptide of the invention and the second portion being, for example, the reporter described above or an immunoglobulin constant region.

The expression systems that may be used for purposes of the invention include, but are not limited to, microorganisms such as bacteria (for example, *E. coli* and *B. subtilis*) transformed with recombinant bacteriophage DNA, plasmid DNA, or cosmid DNA expression vectors containing the nucleic acid molecules of the invention; yeast (for example, *Saccharomyces* and *Pichia*) transformed with recombinant yeast expression vectors containing the nucleic acid molecules of the invention (preferably containing the nucleic acid sequence encoding a polypeptide of the invention); insect cell systems infected with recombinant virus expression vectors (for example, baculovirus) containing the nucleic acid molecules of the invention; plant cell systems infected with recombinant virus expression vectors (for example, cauliflower

19

mosaic virus (CaMV) and tobacco mosaic virus (TMV)) or transformed with recombinant plasmid expression vectors (for example, Ti plasmid) containing nucleotide sequences of nucleic acids of the invention; or mammalian cell systems (for example, COS, CHO, BHK, 293, VERO, HeLa, MDCK, WI38, and NIH 3T3 cells) harboring recombinant expression constructs containing promoters derived from the genome of mammalian cells (for example, the metallothionein promoter) or from mammalian viruses (for example, the adenovirus late promoter and the vaccinia virus 7.5K promoter).

In bacterial systems, a number of expression vectors may be advantageously selected depending upon the use intended for the gene product being expressed. For example, when a large quantity of such a protein is to be produced, for the generation of pharmaceutical compositions containing polypeptides of the invention or for raising antibodies to those polypeptides, vectors that are capable of directing the expression of high levels of fusion protein products that are readily purified may be desirable. Such vectors include, but are not limited to, the *E. coli* expression vector pUR278 (Ruther et al., *EMBO J.* 2:1791, 1983), in which the coding sequence of the insert may be ligated individually into the vector in frame with the lacZ coding region so that a fusion protein is produced; pIN vectors (Inouye and Inouye, *Nucleic Acids Res.* 13:3101-3109, 1985; Van Heeke and Schuster, *J. Biol. Chem.* 264:5503-5509, 1989); and the like. pGEX vectors may also be used to express foreign polypeptides as fusion proteins with glutathione S-transferase (GST). In general, such fusion proteins are soluble and can easily be purified from lysed cells by adsorption to glutathione-agarose beads followed by elution in the presence of free glutathione. The pGEX vectors are designed to include thrombin or factor Xa protease cleavage sites so that the cloned target gene product can be released from the GST moiety.

In an insect system, *Autographa californica* nuclear polyhidrosis virus (AcNPV) can be used as a vector to express foreign genes. The virus grows in *Spodoptera frugiperda* cells. The coding sequence of the insert may be cloned individually into non-essential regions (for example the polyhedrin gene) of the virus and placed under control of an AcNPV promoter (for example the polyhedrin promoter). Successful insertion of the coding sequence will result in inactivation of the polyhedrin gene and production of non-occluded recombinant virus (i.e., virus lacking the proteinaceous coat coded for by the polyhedrin gene). These recombinant viruses are then used

to infect *Spodoptera frugiperda* cells in which the inserted gene is expressed. (for example, see Smith et al., *J. Virol.* 46:584, 1983; Smith, U.S. Patent No. 4,215,051).

In mammalian host cells, a number of viral-based expression systems may be utilized. In cases where an adenovirus is used as an expression vector, the nucleic acid molecule of the invention may be ligated to an adenovirus transcription/translation control complex, for example, the late promoter and tripartite leader sequence. This chimeric gene may then be inserted in the adenovirus genome by *in vitro* or *in vivo* recombination. Insertion in a non-essential region of the viral genome (for example, region E1 or E3) will result in a recombinant virus that is viable and capable of expressing a gene product of the invention in infected hosts (for example, see Logan and Shenk, *Proc. Natl. Acad. Sci. USA* 81:3655-3659, 1984). Specific initiation signals may also be required for efficient translation of inserted nucleic acid molecules. These signals include the ATG initiation codon and adjacent sequences. In cases where an entire gene or cDNA, including its own initiation codon and adjacent sequences, is inserted into the appropriate expression vector, no additional translational control signals may be needed. However, in cases where only a portion of the coding sequence is inserted, exogenous translational control signals, including, perhaps, the ATG initiation codon, must be provided. Furthermore, the initiation codon must be in phase with the reading frame of the desired coding sequence to ensure translation of the entire insert. These exogenous translational control signals and initiation codons can be of a variety of origins, both natural and synthetic. The efficiency of expression may be enhanced by the inclusion of appropriate transcription enhancer elements, transcription terminators, etc. (see Bittner et al., *Methods in Enzymol.* 153:516-544, 1987).

In addition, a host cell strain may be chosen which modulates the expression of the inserted sequences, or modifies and processes the gene product in the specific fashion desired. Such modifications (for example, glycosylation) and processing (for example, cleavage) of protein products may be important for the function of the protein. Different host cells have characteristic and specific mechanisms for the post-translational processing and modification of proteins and gene products. Appropriate cell lines or host systems can be chosen to ensure the correct modification and processing of the foreign protein expressed. To this end, eukaryotic host cells that possess the cellular machinery for proper processing of the primary transcript, glycosylation, and phosphorylation of the gene product may be used. The mammalian cell types listed above are among those that could serve as suitable host cells.

For long-term, high-yield production of recombinant proteins, stable expression is preferred. For example, cell lines that stably express the sequences of nucleic acids or polypeptides of the invention described above may be engineered. Rather than using expression vectors that contain viral origins of replication, host cells can be transformed with DNA controlled by appropriate expression control elements (for example, promoter, enhancer sequences, transcription terminators, polyadenylation sites, etc.), and a selectable marker. Following the introduction of the foreign DNA, engineered cells may be allowed to grow for 1-2 days in an enriched media, and then switched to a selective media. The selectable marker in the recombinant plasmid confers resistance to the selection and allows cells to stably integrate the plasmid into their chromosomes and grow to form foci that in turn can be cloned and expanded into cell lines. This method can advantageously be used to engineer cell lines that express nucleic acids or polypeptides of the invention. Such engineered cell lines may be particularly useful in screening and evaluation of compounds that affect the endogenous activity of the gene product.

A number of selection systems can be used. For example, the herpes simplex virus thymidine kinase (Wigler, et al., *Cell* 11:223, 1977), hypoxanthine-guanine phosphoribosyltransferase (Szybalska and Szybalski, *Proc. Natl. Acad. Sci. USA* 48:2026, 1962), and adenine phosphoribosyltransferase (Lowy, et al., *Cell* 22:817, 1980) genes can be employed in tk⁻, hgprt⁻ or aprt⁻ cells, respectively. Also, anti-metabolite resistance can be used as the basis of selection for the following genes: dhfr, which confers resistance to methotrexate (Wigler et al., *Proc. Natl. Acad. Sci. USA* 77:3567, 1980; O'Hare et al., *Proc. Natl. Acad. Sci. USA* 78:1527, 1981); gpt, which confers resistance to mycophenolic acid (Mulligan and Berg, *Proc. Natl. Acad. Sci. USA* 78:2072, 1981); neo, which confers resistance to the aminoglycoside G-418 (Colberre-Garapin et al., *J. Mol. Biol.* 150:1, 1981); and hygro, which confers resistance to hygromycin (Santerre et al., *Gene* 30:147, 1984).

The nucleic acid molecules of the invention are useful for diagnosis of disorders associated with aberrant expression of nucleic acid molecules of the invention are also useful in genetic mapping and chromosome identification.

Tango-71, Tango-79, and Tango-81 Polypeptides

The invention also includes polypeptides that have a sequence that is substantially identical to the amino acid sequence of Tango-71, Tango-79, or Tango-81 (e.g., polypeptides that are substantially identical to the polypeptide of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:10, SEQ ID NO:12, or SEQ ID NO:14). A polypeptide which is "substantially identical" to a given reference polypeptide is a polypeptide having a sequence that has at least 85%, preferably 90%, and more preferably 95%, 98%, 99% or more identity to the sequence of the given reference polypeptide sequence (e.g., the amino sequence of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:10, SEQ ID NO:12, or SEQ ID NO:14).

The terms "protein" and "polypeptide" are used herein interchangably to describe any chain of amino acids, regardless of length or post-translational modification (for example, glycosylation or phosphorylation). Thus, the term "Tango-71, Tango-79, or Tango-81 polypeptide" includes: full-length, naturally occurring protein of the invention; recombinantly or synthetically produced polypeptide that corresponds to a full-length naturally occurring protein of the invention; or particular domains or portions of the naturally occurring protein. The term also encompasses mature a polypeptide of the invention that has an added amino-terminal methionine (useful for expression in prokaryotic cells).

The polypeptides of the invention described herein are those encoded by any of the nucleic acid molecules described above and include fragments, mutants, truncated forms, and fusion proteins of polypeptides of the invention. These polypeptides can be prepared for a variety of uses, including but not limited to the generation of antibodies, as reagents in diagnostic assays, for the identification of other cellular gene products or compounds that can modulate the activity or expression of nucleic acids or polypeptides of the invention, and as pharmaceutical reagents useful for the treatment of disorders associated with aberrant expression or activity of nucleic acids or polypeptides of the invention.

Preferred polypeptides are substantially pure polypeptides of the invention, including those that correspond to the polypeptide with an intact signal sequence, and the secreted form of the polypeptide. Especially preferred are polypeptides that are soluble under normal physiological conditions.

The invention also encompasses polypeptides that are functionally equivalent to polypeptides of the invention. These polypeptides are equivalent to polypeptides of the

invention in that they are capable of carrying out one or more of the functions of polypeptides of the invention in a biological system. Preferred polypeptides of the invention have 20%, 40%, 50%, 75%, 80%, or even 90% of one or more of the biological activities of the full-length, mature human form of polypeptides of the invention. Such comparisons are generally based on an assay of biological activity in which equal concentrations of the polypeptides are used and compared. The comparison can also be based on the amount of the polypeptide required to reach 50% of the maximal stimulation obtainable.

Functionally equivalent proteins can be those, for example, that contain additional or substituted amino acid residues. Substitutions may be made on the basis of similarity in polarity, charge, solubility, hydrophobicity, hydrophilicity, and/or the amphipathic nature of the residues involved. Amino acids that are typically considered to provide a conservative substitution for one another are specified in the summary of the invention.

Polypeptides that are functionally equivalent to polypeptides of the invention can be made using random mutagenesis techniques well known to those skilled in the art. It is more likely, however, that such polypeptides will be generated by site-directed mutagenesis (again using techniques well known to those skilled in the art). These polypeptides may have increased functionality or decreased functionality.

To design functionally equivalent polypeptides, it is useful to distinguish between conserved positions and variable positions. This can be done by aligning the amino acid sequence of a protein of the invention from one species with its homolog from another species. Skilled artisans will recognize that conserved amino acid residues are more likely to be necessary for preservation of function. Thus, it is preferable that conserved residues are not altered.

Mutations within the coding sequence of nucleic acid molecules of the invention can be made to generate variant genes that are better suited for expression in a selected host cell. For example, N-linked glycosylation sites can be altered or eliminated to achieve, for example, expression of a homogeneous product that is more easily recovered and purified from yeast hosts which are known to hyperglycosylate N-linked sites. To this end, a variety of amino acid substitutions at one or both of the first or third amino acid positions of any one or more of the glycosylation recognition sequences which occur, and/or an amino acid deletion at the second position of any one or more of such recognition sequences, will prevent glycosylation at the modified tripeptide sequence (see, for example, Miyajima et al., *EMBO J.* 5:1193, 1986).

24

The polypeptides of the invention can be expressed fused to another polypeptide, for example, a marker polypeptide or fusion partner. For example, the polypeptide can be fused to a hexa-histidine tag to facilitate purification of bacterially expressed protein or a hemagglutinin tag to facilitate purification of protein expressed in eukaryotic cells.

A fusion protein may be readily purified by utilizing an antibody specific for the fusion protein being expressed. For example, a system described by Janknecht et al. allows for the ready purification of non-denatured fusion proteins expressed in human cell lines (*Proc. Natl. Acad. Sci. USA* 88: 8972-8976, 1991). In this system, the gene of interest is subcloned into a vaccinia recombination plasmid such that the gene's open reading frame is translationally fused to an amino-terminal tag consisting of six histidine residues. Extracts from cells infected with recombinant vaccinia virus are loaded onto Ni^{2+}□nitriloacetic acid-agarose columns and histidine-tagged proteins are selectively eluted with imidazole-containing buffers.

The polypeptides of the invention can be chemically synthesized (for example, see Creighton, "Proteins: Structures and Molecular Principles," W.H. Freeman & Co., NY, 1983), or, perhaps more advantageously, produced by recombinant DNA technology as described herein. For additional guidance, skilled artisans may consult Ausubel et al. (*supra*), Sambrook et al. ("Molecular Cloning, A Laboratory Manual," Cold Spring Harbor Press, Cold Spring Harbor, NY, 1989), and, particularly for examples of chemical synthesis Gait, M.J. Ed. ("Oligonucleotide Synthesis," IRL Press, Oxford, 1984).

The invention also features polypeptides that interact with nucleic acids or polypeptides of the invention (and the genes that encode them) and thereby alter the function of nucleic acids or polypeptides of the invention. Interacting polypeptides can be identified using methods known to those skilled in the art. One suitable method is the "two-hybrid system," which detects protein interactions *in vivo* (Chien et al., *Proc. Natl. Acad. Sci. USA*, 88:9578, 1991). A kit for practicing this method is available from Clontech (Palo Alto, CA).

Transgenic animals

Polypeptides of the invention can also be expressed in transgenic animals. These animals represent a model system for the study of disorders that are caused by or exacerbated by overexpression or underexpression of nucleic acids or polypeptides of the invention, and for the

development of therapeutic agents that modulate the expression or activity of nucleic acids or polypeptides of the invention.

Transgenic animals can be farm animals (pigs, goats, sheep, cows, horses, rabbits, and the like), rodents (such as rats, guinea pigs, and mice), non-human primates (for example, baboons, monkeys, and chimpanzees), and domestic animals (for example, dogs and cats). Transgenic mice are especially preferred.

Any technique known in the art can be used to introduce a Tango-71, Tango-79, or Tango-81 transgene into animals to produce the founder lines of transgenic animals. Such techniques include, but are not limited to, pronuclear microinjection (U.S. Pat. No. 4,873,191); retrovirus mediated gene transfer into germ lines (Van der Putten et al., *Proc. Natl. Acad. Sci.*, *USA* 82:6148, 1985); gene targeting into embryonic stem cells (Thompson et al., *Cell* 56:313, 1989); and electroporation of embryos (Lo, *Mol. Cell. Biol.* 3:1803, 1983).

The present invention provides for transgenic animals that carry a transgene of the invention in all their cells, as well as animals that carry a transgene in some, but not all of their cells. That is, the invention provides for mosaic animals. The transgene can be integrated as a single transgene or in concatamers, e.g., head-to-head tandems or head-to-tail tandems. The transgene can also be selectively introduced into and activated in a particular cell type (Lasko et al., *Proc. Natl. Acad. Sci. USA* 89:6232, 1992). The regulatory sequences required for such a cell-type specific activation will depend upon the particular cell type of interest, and will be apparent to those of skill in the art.

When it is desired that the transgene of the invention be integrated into the chromosomal site of the endogenous gene, gene targeting is preferred. Briefly, when such a technique is to be used, vectors containing some nucleotide sequences homologous to an endogenous gene of the invention are designed for the purpose of integrating, via homologous recombination with chromosomal sequences, into and disrupting the function of the nucleotide sequence of the endogenous gene. The transgene also can be selectively introduced into a particular cell type, thus inactivating the endogenous gene of the invention in only that cell type (Gu et al., *Science* 265:103, 1984). The regulatory sequences required for such a cell-type specific inactivation will depend upon the particular cell type of interest, and will be apparent to those of skill in the art. These techniques are useful for preparing "knock outs" lacking a functional gene.

Once transgenic animals have been generated, the expression of the recombinant gene of the invention can be assayed utilizing standard techniques. Initial screening may be accomplished by Southern blot analysis or PCR techniques to determine whether integration of the transgene has taken place. The level of mRNA expression of the transgene in the tissues of the transgenic animals may also be assessed using techniques which include, but are not limited to, Northern blot analysis of tissue samples obtained from the animal, *in situ* hybridization analysis, and RT-PCR. Biological samples can also be evaluated immunocytochemically using antibodies specific for the product of the transgene of the invention. Samples of tissue expressing the gene of the invention can also be evaluated immunocytochemically using antibodies specific for the product of the transgene of the invention.

For a review of techniques that can be used to generate and assess transgenic animals, skilled artisans can consult Gordon (*Intl. Rev. Cytol.* 115:171-229, 1989), and may obtain additional guidance from, for example: Hogan et al. "Manipulating the Mouse Embryo" (Cold Spring Harbor Press, Cold Spring Harbor, NY, 1986; Krimpenfort et al., *Bio/Technology* 9:86, 1991; Palmiter et al., *Cell* 41:343, 1985; Kraemer et al., "Genetic Manipulation of the Early Mammalian Embryo," Cold Spring Harbor Press, Cold Spring Harbor, NY, 1985; Hammer et al., *Nature* 315:680, 1985; Purcel et al., *Science*, 244:1281, 1986; Wagner et al., U.S. Patent No. 5,175,385; and Krimpenfort et al., U.S. Patent No. 5,175,384 (the latter two publications are hereby incorporated by reference).

Anti- Tango-71, Tango-79, and Tango-81 Antibodies

Human polypeptides of the invention (or immunogenic fragments or analogs) can be used to raise antibodies useful in the invention; such polypeptides can be produced by recombinant techniques or synthesized (see, for example, "Solid Phase Peptide Synthesis," *supra*; Ausubel *et al., supra*). In general, the peptides can be coupled to a carrier protein, such as KLH, as described in Ausubel et al., *supra*, mixed with an adjuvant, and injected into a host mammal. Antibodies can be purified by peptide antigen affinity chromatography.

In particular, various host animals can be immunized by injection with a polypeptide of the invention. Host animals include rabbits, mice, guinea pigs, and rats. Various adjuvants that can be used to increase the immunological response depend on the host species and include Freund's adjuvant (complete and incomplete), mineral gels such as aluminum hydroxide, surface

active substances such as lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, keyhole limpet hemocyanin, and dinitrophenol. Potentially useful human adjuvants include BCG (bacille Calmette-Guerin) and *Corynebacterium parvum*. Polyclonal antibodies are heterogeneous populations of antibody molecules that are contained in the sera of the immunized animals.

Antibodies within the invention therefore include polyclonal antibodies and, in addition, monoclonal antibodies, humanized or chimeric antibodies, single chain antibodies, Fab fragments, F(ab')$_2$ fragments, and molecules produced using a Fab expression library.

Monoclonal antibodies, which are homogeneous populations of antibodies to a particular antigen, can be prepared using the polypeptides of the invention described above and standard hybridoma technology (see, for example, Kohler et al., *Nature* 256:495, 1975; Kohler et al., *Eur. J. Immunol.* 6:511, 1976; Kohler et al., *Eur. J. Immunol.* 6:292, 1976; Hammerling et al., "Monoclonal Antibodies and T Cell Hybridomas," Elsevier, NY, 1981; Ausubel et al., *supra*).

In particular, monoclonal antibodies can be obtained by any technique that provides for the production of antibody molecules by continuous cell lines in culture such as described in Kohler et al., *Nature* 256:495, 1975, and U.S. Patent No. 4,376,110; the human B-cell hybridoma technique (Kosbor et al., *Immunology Today* 4:72, 1983; Cole et al., *Proc. Natl. Acad. Sci. USA* 80:2026, 1983), and the EBV-hybridoma technique (Cole et al., "Monoclonal Antibodies and Cancer Therapy," Alan R. Liss, Inc., pp. 77-96, 1983). Such antibodies can be of any immunoglobulin class including IgG, IgM, IgE, IgA, IgD and any subclass thereof. The hybridoma producing the mAb of this invention may be cultivated *in vitro* or *in vivo*. The ability to produce high titers of mAbs *in vivo* makes this a particularly useful method of production.

Once produced, polyclonal or monoclonal antibodies are tested for specific recognition of polypeptides of the invention by Western blot or immunoprecipitation analysis by standard methods, e.g., as described in Ausubel et al., *supra*. Antibodies that specifically recognize and bind to polypeptides of the invention are useful in the invention. For example, such antibodies can be used in an immunoassay to monitor the level of a polypeptide of the invention produced by a mammal (for example, to determine the amount or subcellular location of a polypeptide of the invention).

Preferably, antibodies of the invention are produced using fragments of the protein of the invention that lie outside highly conserved regions and appear likely to be antigenic, by criteria

such as high frequency of charged residues. In one specific example, such fragments are generated by standard techniques of PCR, and are then cloned into the pGEX expression vector (Ausubel et al., *supra*). Fusion proteins are expressed in *E. coli* and purified using a glutathione agarose affinity matrix as described in Ausubel, *et al.*, *supra*.

In some cases it may be desirable to minimize the potential problems of low affinity or specificity of antisera. In such circumstances, two or three fusions can be generated for each protein, and each fusion can be injected into at least two rabbits. Antisera can be raised by injections in a series, preferably including at least three booster injections.

Antisera may also checked for its ability to immunoprecipitate recombinant proteins of the invention or control proteins, such as glucocorticoid receptor, CAT, or luciferase.

The antibodies can be used, for example, in the detection of the polypeptide of the invention in a biological sample as part of a diagnostic assay. Antibodies also can be used in a screening assay to measure the effect of a candidate compound on expression or localization of a polypeptide of the invention. Additionally, such antibodies can be used in conjunction with the gene therapy techniques described to, for example, evaluate normal and/or genetically engineered cells that express nucleic acids or polypeptides of the invention prior to their introduction into the patient. Such antibodies additionally can be used in a method for inhibiting abnormal activity of nucleic acids or polypeptides of the invention.

In addition, techniques developed for the production of "chimeric antibodies" (Morrison et al., *Proc. Natl. Acad. Sci. USA*, 81:6851, 1984; Neuberger et al., *Nature*, 312:604, 1984; Takeda et al., *Nature*, 314:452, 1984) by splicing the genes from a mouse antibody molecule of appropriate antigen specificity together with genes from a human antibody molecule of appropriate biological activity can be used. A chimeric antibody is a molecule in which different portions are derived from different animal species, such as those having a variable region derived from a murine mAb and a human immunoglobulin constant region.

Generally, partially human antibodies and fully human antibodies have a longer half-life within the human body than other antibodies. Accordingly, lower dosages and less frequent administration are often possible. Modifications such as lipidation can be used to stabilize antibodies and to enhance uptake and tissue penetration (e.g., into the brain). A method for lipidation of antibodies is described by Cruikshank et al. ((1997) J. Acquired Immune Deficiency Syndromes and Human Retrovirology 14:193).

Alternatively, techniques described for the production of single chain antibodies (U.S. Patent Nos. 4,946,778, 4,946,778, and 4,704,692) can be adapted to produce single chain antibodies against polypeptides of the invention. Single chain antibodies are formed by linking the heavy and light chain fragments of the Fv region via an amino acid bridge, resulting in a single chain polypeptide.

Antibody fragments that recognize and bind to specific epitopes can be generated by known techniques. For example, such fragments include but are not limited to F(ab')$_2$ fragments that can be produced by pepsin digestion of the antibody molecule, and Fab fragments that can be generated by reducing the disulfide bridges of F(ab')$_2$ fragments. Alternatively, Fab expression libraries can be constructed (Huse et al., *Science*, 246:1275, 1989) to allow rapid and easy identification of monoclonal Fab fragments with the desired specificity.

Antibodies to polypeptides of the invention can, in turn, be used to generate anti-idiotype antibodies that resemble a portion of the protein of the invention using techniques well known to those skilled in the art (see, e.g., Greenspan et al., *FASEB J.* 7:437, 1993; Nissinoff, *J. Immunol.* 147:2429, 1991). For example, antibodies that bind to the protein of the invention and competitively inhibit the binding of a binding partner of the protein can be used to generate anti-idiotypes that resemble a binding partner binding domain of the protein and, therefore, bind and neutralize a binding partner of the protein. Such neutralizing anti-idiotypic antibodies or Fab fragments of such anti-idiotypic antibodies can be used in therapeutic regimens.

Antibodies can be humanized by methods known in the art. For example, monoclonal antibodies with a desired binding specificity can be commercially humanized (Scotgene, Scotland; Oxford Molecular, Palo Alto, CA). Fully human antibodies, such as those expressed in transgenic animals are also features of the invention (Green et al., *Nature Genetics* 7:13-21, 1994; see also U.S. Patents 5,545,806 and 5,569,825, both of which are hereby incorporated by reference).

The methods described herein in which anti-polypeptide-of-the-invention antibodies are employed may be performed, for example, by utilizing pre-packaged diagnostic kits comprising at least one specific polypeptide-of-the-invention antibody reagent described herein, which may be conveniently used, for example, in clinical settings, to diagnose patients exhibiting symptoms of disorders associated with aberrant expression of nucleic acids or polypeptides of the invention.

An antibody (or fragment thereof) can be conjugated to a therapeutic moiety such as a cytotoxin, a therapeutic agent, or a radioactive agent (e.g., a radioactive metal ion). Cytotoxins and cytotoxic agents include any agent that is detrimental to cells. Examples of such agents include taxol, cytochalasin B, gramicidin D, ethidium bromide, emetine, mitomycin, etoposide, tenoposide, vincristine, vinblastine, colchicin, doxorubicin, daunorubicin, dihydroxy anthracin dione, mitoxantrone, mithramycin, actinomycin D, 1-dehydrotestosterone, glucocorticoids, procaine, tetracaine, lidocaine, propranolol, and puromycin and analogs or homologs thereof. Therapeutic agents include, but are not limited to, antimetabolites (e.g., methotrexate, 6-mercaptopurine, 6-thioguanine, cytarabine, and 5-fluorouracil decarbazine), alkylating agents (e.g., mechlorethamine, thioepa chlorambucil, melphalan, carmustine (BSNU) and lomustine (CCNU), cyclothosphamide, busulfan, dibromomannitol, streptozotocin, mitomycin C, and cis-dichlorodiamine platinum (II) (DDP) cisplatin), anthracyclines (e.g., daunorubicin {formerly designated daunomycin} and doxorubicin), antibiotics (e.g., dactinomycin {formerly designated actinomycin}, bleomycin, mithramycin, and anthramycin), and anti-mitotic agents (e.g., vincristine and vinblastine).

Conjugated antibodies of the invention can be used for modifying a given biological response, the drug moiety not being limited to classical chemical therapeutic agents. For example, the drug moiety can be a protein or polypeptide possessing a desired biological activity. Such proteins include, for example, toxins such as abrin, ricin A, Pseudomonas exotoxin, or diphtheria toxin; proteins such as tumor necrosis factor, alpha-interferon, beta-interferon, nerve growth factor, platelet derived growth factor, tissue plasminogen activator; and biological response modifiers such as lymphokines, interleukin-1, interleukin-2, interleukin-6, granulocyte macrophage colony stimulating factor, granulocyte colony stimulating factor, or other growth factors.

Techniques for conjugating a therapeutic moiety to an antibody are well known (see, e.g., Arnon et al., 1985, "Monoclonal Antibodies For Immunotargeting Of Drugs In Cancer Therapy", in Monoclonal Antibodies And Cancer Therapy, Reisfeld et al., Eds., Alan R. Liss, Inc. pp. 243-256; Hellstrom et al., 1987, "Antibodies For Drug Delivery", in Controlled Drug Delivery, 2nd ed., Robinson et al., Eds., Marcel Dekker, Inc., pp. 623-653; Thorpe, 1985, "Antibody Carriers Of Cytotoxic Agents In Cancer Therapy: A Review", in Monoclonal Antibodies '84: Biological And Clinical Applications, Pinchera et al., Eds., pp. 475-506; "Analysis, Results, And Future

Prospective Of The Therapeutic Use Of Radiolabeled Antibody In Cancer Therapy", in Monoclonal Antibodies For Cancer Detection And Therapy, Baldwin et al., Eds., Academic Press, pp. 303-316, 1985; and Thorpe et al., 1982, Immunol. Rev., 62:119-158). Alternatively, an antibody can be conjugated to a second antibody to form an antibody heteroconjugate as described by Segal in U.S. Patent No. 4,676,980.

Antisense Nucleic Acids

Treatment regimes based on an "antisense" approach involve the design of oligonucleotides (either DNA or RNA) that are complementary to mRNA of the invention. These oligonucleotides bind to the complementary mRNA transcripts of the invention and prevent translation. Absolute complementarity, although preferred, is not required. A sequence "complementary" to a portion of an RNA, as referred to herein, means a sequence having sufficient complementarily to be able to hybridize with the RNA, forming a stable duplex; in the case of double-stranded antisense nucleic acids, a single strand of the duplex DNA may be tested, or triplex formation may be assayed. The ability to hybridize will depend on both the degree of complementarily and the length of the antisense nucleic acid. Generally, the longer the hybridizing nucleic acid, the more base mismatches with an RNA it may contain and still form a stable duplex (or triplex, as the case may be). One skilled in the art can ascertain a tolerable degree of mismatch by use of standard procedures to determine the melting point of the hybridized complex.

Oligonucleotides that are complementary to the 5' end of the message, e.g., the 5' untranslated sequence up to and including the AUG initiation codon, should work most efficiently at inhibiting translation. However, sequences complementary to the 3' untranslated sequences of mRNAs recently have been shown to be effective at inhibiting translation of mRNAs as well (Wagner, *Nature* 372:333, 1984). Thus, oligonucleotides complementary to either the 5' or 3' non-translated, non-coding regions of the gene or mRNA could be used in an antisense approach to inhibit translation of endogenous mRNA. Oligonucleotides complementary to the 5' untranslated region of the mRNA should include the complement of the AUG start codon.

Antisense oligonucleotides complementary to mRNA coding regions are less efficient inhibitors of translation but could be used in accordance with the invention. Whether designed to

32

hybridize to the 5', 3', or coding region of an mRNA, antisense nucleic acids should be at least six nucleotides in length, and are preferably oligonucleotides ranging from 6 to about 50 nucleotides in length. In specific aspects the oligonucleotide is at least 10 nucleotides, at least 17 nucleotides, at least 25 nucleotides, or at least 50 nucleotides.

Regardless of the choice of target sequence, it is preferred that *in vitro* studies are first performed to quantitate the ability of the antisense oligonucleotide to inhibit gene expression. It is preferred that these studies utilize controls that distinguish between antisense gene inhibition and nonspecific biological effects of oligonucleotides. It is also preferred that these studies compare levels of the target RNA or protein with that of an internal control RNA or protein. Additionally, it is envisioned that results obtained using the antisense oligonucleotide are compared with those obtained using a control oligonucleotide. It is preferred that the control oligonucleotide is of approximately the same length as the test oligonucleotide and that the nucleotide sequence of the oligonucleotide differs from the antisense sequence no more than is necessary to prevent specific hybridization to the target sequence.

The oligonucleotides can be DNA or RNA or chimeric mixtures or derivatives or modified versions thereof, single-stranded or double-stranded. The oligonucleotide can be modified at the base moiety, sugar moiety, or phosphate backbone, for example, to improve stability of the molecule, hybridization, etc. The oligonucleotide may include other appended groups such as peptides (e.g., for targeting host cell receptors *in vivo*), or agents facilitating transport across the cell membrane (as described, e.g., in Letsinger et al., *Proc. Natl. Acad. Sci. USA* 86:6553, 1989; Lemaitre et al., *Proc. Natl. Acad. Sci. USA* 84:648, 1987; PCT Publication No. WO 88/09810) or the blood-brain barrier (see, for example, PCT Publication No. WO 89/10134), or hybridization-triggered cleavage agents (see, for example, Krol et al., *BioTechniques* 6:958, 1988), or intercalating agents (see, for example, Zon, *Pharm. Res.* 5:539, 1988). To this end, the oligonucleotide can be conjugated to another molecule, e.g., a peptide, hybridization triggered cross-linking agent, transport agent, or hybridization-triggered cleavage agent.

The antisense oligonucleotide may comprise at least one modified base moiety which is selected from the group including, but not limited to, 5-fluorouracil, 5-bromouracil, 5-chlorouracil, 5-iodouracil, hypoxanthine, xantine, 4-acetylcytosine, 5-(carboxyhydroxylmethyl) uracil, 5-carboxymethylaminomethyl-2-thiouridine, 5-carboxymethyl-

antisense DNA or RNA to cells; e.g., antisense molecules can be injected directly into the tissue site, or modified antisense molecules, designed to target the desired cells (e.g., antisense linked to peptides or antibodies that specifically bind receptors or antigens expressed on the target cell surface) can be administered systemically.

However, it is often difficult to achieve intracellular concentrations of the antisense molecule sufficient to suppress translation of endogenous mRNAs. Therefore, a preferred approach uses a recombinant DNA construct in which the antisense oligonucleotide is placed under the control of a strong *pol* III or *pol* II promoter. The use of such a construct to transfect target cells in the patient will result in the transcription of sufficient amounts of single stranded RNAs that will form complementary base pairs with the endogenous transcripts of nucleic acids of the invention and thereby prevent translation of the endogenous mRNA. For example, a vector can be introduced *in vivo* such that it is taken up by a cell and directs the transcription of an antisense RNA. Such a vector can remain episomal or become chromosomally integrated, as long as it can be transcribed to produce the desired antisense RNA.

Such vectors can be constructed by recombinant DNA technology methods standard in the art. Vectors can be plasmid, viral, or others known in the art, used for replication and expression in mammalian cells. Expression of the sequence encoding the antisense RNA can be by any promoter known in the art to act in mammalian, preferably human cells. Such promoters can be inducible or constitutive. Such promoters include, but are not limited to: the SV40 early promoter region (Bernoist et al., *Nature* 290:304, 1981); the promoter contained in the 3' long terminal repeat of Rous sarcoma virus (Yamamoto et al., *Cell* 22:787-797, 1988); the herpes thymidine kinase promoter (Wagner et al., *Proc. Natl. Acad. Sci. USA* 78:1441, 1981); or the regulatory sequences of the metallothionein gene (Brinster et al., *Nature* 296:39, 1988).

Ribozymes

Ribozyme molecules designed to catalytically cleave mRNA transcripts of nucleic acids of the invention can be used to prevent translation and expression of mRNA of the invention. (see, e.g., PCT Publication WO 90/11364; Saraver et al., *Science* 247:1222, 1990). While various ribozymes that cleave mRNA at site-specific recognition sequences can be used to destroy mRNAs of the invention, the use of hammerhead ribozymes is preferred. Hammerhead ribozymes cleave mRNAs at locations dictated by flanking regions that form complementary

base pairs with the target mRNA. The sole requirement is that the target mRNA have the following sequence of two bases: 5'-UG-3'. The construction and production of hammerhead ribozymes is well known in the art (Haseloff et al., *Nature* 334:585, 1988). There are numerous examples of potential hammerhead ribozyme cleavage sites within the nucleotide sequence of human cDNA of the invention. Preferably, the ribozyme is engineered so that the cleavage recognition site is located near the 5' end of the mRNA, i.e., to increase efficiency and minimize the intracellular accumulation of non-functional mRNA transcripts.

The ribozymes of the present invention also include RNA endoribonucleases (hereinafter "Cech-type ribozymes"), such as the one that occurs naturally in *Tetrahymena thermophila* (known as the IVS or L-19 IVS RNA), and which has been extensively described by Cech and his collaborators (Zaug et al., *Science* 224:574, 1984; Zaug et al., *Science*, 231:470, 1986; Zug et al., *Nature* 324:429, 1986; PCT Application No. WO 88/04300; and Been et al., *Cell* 47:207, 1986). The Cech-type ribozymes have an eight base-pair sequence that hybridizes to a target RNA sequence, whereafter cleavage of the target RNA takes place. The invention encompasses those Cech-type ribozymes that target eight base-pair active site sequences present in nucleic acids of the invention.

As in the antisense approach, the ribozymes can be composed of modified oligonucleotides (e.g., for improved stability, targeting, etc.), and should be delivered to cells which express nucleic acids or polypeptides of the invention *in vivo*. A preferred method of delivery involves using a DNA construct "encoding" the ribozyme under the control of a strong constitutive *pol* III or *pol* II promoter, so that transfected cells will produce sufficient quantities of the ribozyme to destroy endogenous messages and inhibit translation. Because ribozymes, unlike antisense molecules, are catalytic, a lower intracellular concentration is required for efficiency.

Other Methods for Modulating Tango-71, Tango-79, or Tango-81 Expression

Endogenous expression of a gene of the invention can also be modulated by inactivating the endogenous gene or its promoter using targeted homologous recombination (see, e.g., U.S. Patent No. 5,464,764). For example, a mutant, non-functional gene of the invention (or a completely unrelated DNA sequence) flanked by DNA homologous to the endogenous gene of the invention (either the coding regions or regulatory regions of the gene of the invention) can be

36

used, with or without a selectable marker and/or a negative selectable marker, to transfect cells that express the endogenous gene of the invention *in vivo*. Insertion of the DNA construct, via targeted homologous recombination, results in inactivation of the gene of the invention. Such approaches are particularly suited for use in the agricultural field where modifications to ES (embryonic stem) cells can be used to generate animal offspring with an inactive gene of the invention. However, this approach can be adapted for use in humans, provided the recombinant DNA constructs are directly administered or targeted to the required site *in vivo* using appropriate viral vectors.

Alternatively, endogenous expression of a gene of the invention can be modulated by targeting deoxyribonucleotide sequences complementary to the regulatory region of the gene of the invention (*i.e.*, the promoter and/or enhancers of a gene of the invention) to form triple helical structures that prevent transcription of the gene of the invention in target cells in the body (Helene, *Anticancer Drug Res.* 6:569, 1981; Helene et al., *Ann. N.Y. Acad. Sci.* 660:27, 1992; and Maher, *Bioassays* 14:807, 1992).

The invention includes methods for preparing pharmaceutical compositions for modulating the expression or activity of a polypeptide or nucleic acid of the invention. Such methods comprise formulating a pharmaceutically acceptable carrier with an agent which modulates expression or activity of a polypeptide or nucleic acid of the invention. Such compositions can further include additional active agents. Thus, the invention further includes methods for preparing a pharmaceutical composition by formulating a pharmaceutically acceptable carrier with an agent that modulates expression or activity of a polypeptide or nucleic acid of the invention and one or more additional active compounds.

The agent that modulates expression or activity can, for example, be a small molecule. For example, such small molecules include peptides, peptidomimetics, amino acids, amino acid analogs, polynucleotides, polynucleotide analogs, nucleotides, nucleotide analogs, organic or inorganic compounds (i.e., including heteroorganic and organometallic compounds) having a molecular weight less than about 10,000 grams per mole, organic or inorganic compounds having a molecular weight less than about 5,000 grams per mole, organic or inorganic compounds having a molecular weight less than about 1,000 grams per mole, organic or inorganic compounds having a molecular weight less than about 500 grams per mole, and salts, esters, and other pharmaceutically acceptable forms of such compounds.

It is understood that appropriate doses of small molecule agents and protein or polypeptide agents depends upon a number of factors within the ken of the ordinarily skilled physician, veterinarian, or researcher. The dose(s) of these agents will vary, for example, depending upon the identity, size, and condition of the subject or sample being treated, further depending upon the route by which the composition is to be administered, if applicable, and the effect which the practitioner desires the agent to have upon the nucleic acid or polypeptide of the invention. Examples of doses of a small molecule include milligram or microgram amounts per kilogram of subject or sample weight (e.g., about 1 microgram per kilogram to about 500 milligrams per kilogram, about 100 micrograms per kilogram to about 5 milligrams per kilogram, or about 1 microgram per kilogram to about 50 micrograms per kilogram). Examples of doses of a protein or polypeptide include gram, milligram or microgram amounts per kilogram of subject or sample weight (e.g., about 1 microgram per kilogram to about 5 grams per kilogram, about 100 micrograms per kilogram to about 500 milligrams per kilogram, or about 1 milligram per kilogram to about 50 milligrams per kilogram). For antibodies, examples of dosages are from about 0.1 milligram per kilogram to 100 milligrams per kilogram of body weight (generally 10 milligrams per kilogram to 20 milligrams per kilogram). If the antibody is to act in the brain, a dosage of 50 milligrams per kilogram to 100 milligrams per kilogram is usually appropriate. It is furthermore understood that appropriate doses of one of these agents depend upon the potency of the agent with respect to the expression or activity to be modulated. Such appropriate doses can be determined using the assays described herein. When one or more of these agents is to be administered to an animal (e.g., a human) in order to modulate expression or activity of a polypeptide or nucleic acid of the invention, a physician, veterinarian, or researcher can, for example, prescribe a relatively low dose at first, subsequently increasing the dose until an appropriate response is obtained. In addition, it is understood that the specific dose level for any particular animal subject will depend upon a variety of factors including the activity of the specific agent employed, the age, body weight, general health, gender, and diet of the subject, the time of administration, the route of administration, the rate of excretion, any drug combination, and the degree of expression or activity to be modulated.

As an alternative to making determinations based on the absolute expression level of selected genes, determinations may be based on the normalized expression levels of these genes. Expression levels are normalized by correcting the absolute expression level of a gene encoding

a polypeptide of the invention by comparing its expression to the expression of a different gene. e.g., a housekeeping gene that is constitutively expressed. Suitable genes for normalization include housekeeping genes such as the actin gene. This normalization allows the comparison of the expression level in one sample (e.g., a patient sample), to another sample, or between samples from different sources.

Alternatively, the expression level can be provided as a relative expression level. To determine a relative expression level of a gene, the level of expression of the gene is determined for 10 or more samples of different endothelial (e.g. intestinal endothelium, airway endothelium, or other mucosal epithelium) cell isolates, preferably 50 or more samples, prior to the determination of the expression level for the sample in question. The mean expression level of each of the genes assayed in the larger number of samples is determined and this is used as a baseline expression level for the gene(s) in question. The expression level of the gene determined for the test sample (absolute level of expression) is then divided by the mean expression value obtained for that gene. This provides a relative expression level and aids in identifying extreme cases of disorders associated with aberrant expression of a gene encoding a polypeptide of the invention protein or with aberrant expression of a ligand thereof.

Preferably, the samples used in the baseline determination will be from either or both of cells which aberrantly express a gene encoding a polypeptide of the invention or a ligand thereof (i.e. 'diseased cells') and cells which express a gene encoding a polypeptide of the invention at a normal levelor a ligand thereof (i.e. 'normal' cells). The choice of the cell source is dependent on the use of the relative expression level. Using expression found in normal tissues as a mean expression score aids in validating whether aberrance in expression of a gene encoding a polypeptide of the invention occurs specifically in diseased cells. Such a use is particularly important in identifying whether a gene encoding a polypeptide of the invention can serve as a target gene. In addition, as more data is accumulated, the mean expression value can be revised, providing improved relative expression values based on accumulated data. Expression data from endothelial cells (e.g. mucosal endothelial cells) provides a means for grading the severity of the disorder.

Detecting Proteins Associated with Tango-71, Tango-79, or Tango-81

The invention also features polypeptides that interact with Tango-71, Tango-79, or Tango-81. Any method suitable for detecting protein-protein interactions may be employed for identifying transmembrane proteins, intracellular, or extracellular proteins that interact with polypeptides of the invention. Among the traditional methods which may be employed are co-immunoprecipitation, cross-linking and co-purification through gradients or chromatographic columns of cell lysates or proteins obtained from cell lysates and the use of polypeptides of the invention to identify proteins in the lysate that interact with polypeptides of the invention. For these assays, the polypeptide of the invention can be full length polypeptide of the invention, a soluble extracellular domain of a polypeptide of the invention, or some other suitable polypeptide of the invention. Once isolated, such an interacting protein can be identified and cloned and then used, in conjunction with standard techniques, to identify proteins with which it interacts. For example, at least a portion of the amino acid sequence of a protein which interacts with the polypeptide of the invention can be ascertained using techniques well known to those of skill in the art, such as via the Edman degradation technique. The amino acid sequence obtained may be used as a guide for the generation of oligonucleotide mixtures that can be used to screen for gene sequences encoding the interacting protein. Screening may be accomplished, for example, by standard hybridization or PCR techniques. Techniques for the generation of oligonucleotide mixtures and the screening are well-known. (Ausubel, *supra;* and "PCR Protocols: A Guide to Methods and Applications," Innis et al., eds. Academic Press, Inc., NY, 1990).

Additionally, methods may be employed which result directly in the identification of genes which encode proteins which interact with polypeptides of the invention. These methods include, for example, screening expression libraries, in a manner similar to the well known technique of antibody probing of λgt11 libraries, using labeled polypeptide of the invention or a fusion protein of the invention, e.g., a polypeptide of the invention or domain thereof fused to a marker such as an enzyme, fluorescent dye, a luminescent protein, or to an IgFc domain.

There are also methods capable of detecting protein interaction. A method that detects protein interactions *in vivo* is the two-hybrid system (Chien et al., *Proc. Natl. Acad. Sci. USA,* 88:9578, 1991). A kit for practicing this method is available from Clontech (Palo Alto, CA).

Identification of Tango-71, Tango-79, or Tango-81 Receptors

Receptors of polypeptides of the invention can be identified as follows. First cells or tissues that bind polypeptides of the invention are identified. An expression library is prepared using mRNA isolated from cells that bind to polypeptides of the invention. The expression library is used to transfect; eukaryotic cells, e.g., CHO cells. Detectably labeled polypeptides of the invention are used to identify clones that bind polypeptides of the invention. These clones are isolated and purified. The expression plasmid is then isolated from polypeptides-of-the-invention-binding clones. These expression plasmids will encode putative receptors of polypeptides of the invention.

Cells or tissues bearing a receptor of a polypeptide of the invention can be identified by exposing detectably labeled polypeptide of the invention to various cells lines and tissues. Alternatively a microphysiometer can be used to determine whether a selected cells responds to the presence of a cell receptor ligand (McConnel et al., *Science* 257:1906, 1992).

Compounds that bind Tango-71, Tango-79, or Tango-81

Compounds that bind nucleic acids or polypeptides of the invention can be identified using any standard binding assay. For example, candidate compounds can be bound to a solid support. A nucleic acid or polypeptide of the invention is then exposed to the immobilized compound and binding is measured (European Patent Application 84/03564).

In one embodiment, the invention provides assays for screening candidate or test compounds that bind with or modulate the activity of the membrane-bound form of a polypeptide of the invention or biologically active portion thereof. The test compounds of the present invention can be obtained using any of the numerous approaches in combinatorial library methods known in the art, including: biological libraries; spatially addressable parallel solid phase or solution phase libraries; synthetic library methods requiring deconvolution; the "one-bead one-compound" library method; and synthetic library methods using affinity chromatography selection. The biological library approach is limited to peptide libraries, while the other four approaches are applicable to peptide, non-peptide oligomer, or small molecule libraries of compounds (Lam (1997) Anticancer Drug Des. 12:145).

Examples of methods useful for the synthesis of molecular libraries can be found in the art, for example in: DeWitt et al. (1993) Proc. Natl. Acad. Sci. USA 90:6909; Erb et al. (1994)

Proc. Natl. Acad. Sci. USA 91:11422; Zuckermann et al. (1994). J. Med. Chem. 37:2678; Cho et al. (1993) Science 261:1303; Carrell et al. (1994) Angew. Chem. Int. Ed. Engl. 33:2059; Carell et al. (1994) Angew. Chem. Int. Ed. Engl. 33:2061; and Gallop et al. (1994) J. Med. Chem. 37:1233.

Libraries of compounds can be presented in solution (e.g., Houghten (1992) Bio/Techniques 13:412-421), or on beads (Lam (1991) Nature 354:82-84), chips (Fodor (1993) Nature 364:555-556), bacteria (U.S. Patent No. 5,223,409), spores (Patent numbers 5,571,698: 5,403,484; and 5,223,409), plasmids (Cull et al. (1992) Proc. Natl. Acad. Sci. USA 89:1865-1869) or phage (Scott and Smith (1990) Science 249:386-390; Devlin (1990) Science 249:404-406; Cwirla et al. (1990) Proc. Natl. Acad. Sci. USA 87:6378-6382; and Felici (1991) J. Mol. Biol. 222:301-310).

In one embodiment, an assay is a cell-based assay in which a cell that expresses a membrane-bound form of a polypeptide of the invention, or a biologically active portion thereof, on the cell surface is contacted with a test compound and the ability of the test compound to bind with the polypeptide is determined. The cell, for example, can be a yeast cell or a cell of mammalian origin. Determining the ability of the test compound to bind with the polypeptide can be accomplished, for example, by coupling the test compound with a radioisotope or enzymatic label such that binding of the test compound to the polypeptide or biologically active portion thereof can be determined by detecting the labeled compound in a complex. For example, test compounds can be labeled with ^{125}I, ^{35}S, ^{14}C, or ^{3}H, either directly or indirectly, and the radioisotope detected by direct counting of radio-emission or by scintillation counting. Alternatively, test compounds can be enzymatically labeled with, for example, horseradish peroxidase, alkaline phosphatase, or luciferase, and the enzymatic label detected by determination of conversion of an appropriate substrate to product. In one embodiment, the assay comprises contacting a cell which expresses a membrane-bound form of a polypeptide of the invention, or a biologically active portion thereof, on the cell surface with a known compound that binds the polypeptide to form an assay mixture, contacting the assay mixture with a test compound, and determining the ability of the test compound to interact with the polypeptide, wherein determining the ability of the test compound to interact with the polypeptide comprises determining the ability of the test compound to preferentially bind with the polypeptide or a biologically active portion thereof as compared to the known compound.

In another embodiment, the assay involves assessment of an activity characteristic of the polypeptide, wherein binding of the test compound with the polypeptide or a biologically active portion thereof alters (i.e., increases or decreases) the activity of the polypeptide.

Uses and Methods of the Invention

The nucleic acid molecules, proteins, protein homologs, and antibodies described herein can be used in one or more of the following methods: a) screening assays; b) detection assays (e.g., chromosomal mapping, tissue typing, forensic biology); c) predictive medicine (e.g., diagnostic assays, prognostic assays, monitoring clinical trials, and pharmacogenomics); and d) methods of treatment (e.g., therapeutic and prophylactic). For example, polypeptides of the invention can to used to (i) modulate cellular proliferation; (ii) modulate cellular differentiation; and/or (iii) modulate cellular adhesion. The isolated nucleic acid molecules of the invention can be used to express proteins (e.g., via a recombinant expression vector in a host cell in gene therapy applications), to detect mRNA (e.g., in a biological sample) or a genetic lesion, and to modulate activity of a polypeptide of the invention. In addition, the polypeptides of the invention can be used to screen drugs or compounds which modulate activity or expression of a polypeptide of the invention as well as to treat disorders characterized by insufficient or excessive production of a protein of the invention or production of a form of a protein of the invention which has decreased or aberrant activity compared to the wild type protein. In addition, the antibodies of the invention can be used to detect and isolate a protein of the and modulate activity of a protein of the invention.

This invention further pertains to novel agents identified by the above-described screening assays and uses thereof for treatments as described herein.

A. Screening Assays

The invention provides a method (also referred to herein as a Ascreening assay@) for identifying modulators, i.e., candidate or test compounds or agents (e.g., peptides, peptidomimetics, small molecules or other drugs) which bind to polypeptide of the invention or have a stimulatory or inhibitory effect on, for example, expression or activity of a polypeptide of the invention.

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In one embodiment, the invention provides assays for screening candidate or test compounds that bind to or modulate the activity of the membrane-bound form of a polypeptide of the invention or biologically active portion thereof. The test compounds of the present invention can be obtained using any of the numerous approaches in combinatorial library methods known in the art, including: biological libraries; spatially addressable parallel solid phase or solution phase libraries; synthetic library methods requiring deconvolution; the Aone-bead one-compound@ library method; and synthetic library methods using affinity chromatography selection. The biological library approach is limited to peptide libraries, while the other four approaches are applicable to peptide, non-peptide oligomer or small molecule libraries of compounds (Lam (1997) *Anticancer Drug Des.* 12:145).

Examples of methods for the synthesis of molecular libraries can be found in the art, for example in: DeWitt et al. (1993) *Proc. Natl. Acad. Sci. USA* 90:6909; Erb et al. (1994) *Proc. Natl. Acad. Sci. USA* 91:11422; Zuckermann et al. (1994). *J. Med. Chem.* 37:2678; Cho et al. (1993) *Science* 261:1303; Carrell et al. (1994) *Angew. Chem. Int. Ed. Engl.* 33:2059; Carell et al. (1994) *Angew. Chem. Int. Ed. Engl.* 33:2061; and Gallop et al. (1994) *J. Med. Chem.* 37:1233.

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In one embodiment, an assay is a cell-based assay in which a cell that expresses a membrane-bound form of a polypeptide of the invention, or a biologically active portion thereof, on the cell surface is contacted with a test compound and the ability of the test compound to bind to the polypeptide determined. The cell, for example, can be a yeast cell or a cell of mammalian origin. Determining the ability of the test compound to bind to the polypeptide can be accomplished, for example, by coupling the test compound with a radioisotope or enzymatic label such that binding of the test compound to the polypeptide or biologically active portion thereof can be determined by detecting the labeled compound in a complex. For example, test compounds can be labeled with ^{125}I, ^{35}S, ^{14}C, or ^3H, either directly or indirectly, and the

44

radioisotope detected by direct counting of radioemmission or by scintillation counting. Alternatively, test compounds can be enzymatically labeled with, for example, horseradish peroxidase, alkaline phosphatase, or luciferase, and the enzymatic label detected by determination of conversion of an appropriate substrate to product. In a preferred embodiment. the assay comprises contacting a cell which expresses a membrane-bound form of a polypeptide of the invention, or a biologically active portion thereof, on the cell surface with a known compound which binds the polypeptide to form an assay mixture, contacting the assay mixture with a test compound, and determining the ability of the test compound to interact with the polypeptide, wherein determining the ability of the test compound to interact with the polypeptide comprises determining the ability of the test compound to preferentially bind to the polypeptide or a biologically active portion thereof as compared to the known compound.

In another embodiment, an assay is a cell-based assay comprising contacting a cell expressing a membrane-bound form of a polypeptide of the invention, or a biologically active portion thereof, on the cell surface with a test compound and determining the ability of the test compound to modulate (e.g., stimulate or inhibit) the activity of the polypeptide or biologically active portion thereof. Determining the ability of the test compound to modulate the activity of the polypeptide or a biologically active portion thereof can be accomplished, for example, by determining the ability of the polypeptide protein to bind to or interact with a target molecule.

Determining the ability of a polypeptide of the invention to bind to or interact with a target molecule can be accomplished by one of the methods described above for determining direct binding. As used herein, a "target molecule" is a molecule with which a selected polypeptide (e.g., a polypeptide of the invention) binds or interacts with in nature, for example, a molecule on the surface of a cell which expresses the selected protein, a molecule on the surface of a second cell, a molecule in the extracellular milieu, a molecule associated with the internal surface of a cell membrane or a cytoplasmic molecule. A target molecule can be a polypeptide of the invention or some other polypeptide or protein. For example, a target molecule can be a component of a signal transduction pathway which facilitates transduction of an extracellular signal (e.g., a signal generated by binding of a compound to a polypeptide of the invention) through the cell membrane and into the cell or a second intercellular protein which has catalytic activity or a protein which facilitates the association of downstream signaling molecules with a polypeptide of the invention. Determining the ability of a polypeptide of the invention to bind to

or interact with a target molecule can be accomplished by determining the activity of the target molecule. For example, the activity of the target molecule can be determined by detecting induction of a cellular second messenger of the target (*e.g.*, intracellular Ca^{2+}, diacylglycerol, IP3, etc.), detecting catalytic/enzymatic activity of the target on an appropriate substrate, detecting the induction of a reporter gene (*e.g.*, a regulatory element that is responsive to a polypeptide of the invention operably linked to a nucleic acid encoding a detectable marker, *e.g.*, luciferase), or detecting a cellular response, for example, cellular differentiation, or cell proliferation.

In yet another embodiment, an assay of the present invention is a cell-free assay comprising contacting a polypeptide of the invention or biologically active portion thereof with a test compound and determining the ability of the test compound to bind to the polypeptide or biologically active portion thereof. Binding of the test compound to the polypeptide can be determined either directly or indirectly as described above. In a preferred embodiment, the assay includes contacting the polypeptide of the invention or biologically active portion thereof with a known compound which binds the polypeptide to form an assay mixture, contacting the assay mixture with a test compound, and determining the ability of the test compound to interact with the polypeptide, wherein determining the ability of the test compound to interact with the polypeptide comprises determining the ability of the test compound to preferentially bind to the polypeptide or biologically active portion thereof as compared to the known compound.

In another embodiment, an assay is a cell-free assay comprising contacting a polypeptide of the invention or biologically active portion thereof with a test compound and determining the ability of the test compound to modulate (*e.g.*, stimulate or inhibit) the activity of the polypeptide or biologically active portion thereof. Determining the ability of the test compound to modulate the activity of the polypeptide can be accomplished, for example, by determining the ability of the polypeptide to bind to a target molecule by one of the methods described above for determining direct binding. In an alternative embodiment, determining the ability of the test compound to modulate the activity of the polypeptide can be accomplished by determining the ability of the polypeptide of the invention to further modulate the target molecule. For example, the catalytic/enzymatic activity of the target molecule on an appropriate substrate can be determined as previously described.

In yet another embodiment, the cell-free assay comprises contacting a polypeptide of the invention or biologically active portion thereof with a known compound which binds the polypeptide to form an assay mixture, contacting the assay mixture with a test compound, and determining the ability of the test compound to interact with the polypeptide, wherein determining the ability of the test compound to interact with the polypeptide comprises determining the ability of the polypeptide to preferentially bind to or modulate the activity of a target molecule.

The cell-free assays of the present invention are amenable to use of both a soluble form or the membrane-bound form of a polypeptide of the invention. In the case of cell-free assays comprising the membrane-bound form of the polypeptide, it may be desirable to utilize a solubilizing agent such that the membrane-bound form of the polypeptide is maintained in solution. Examples of such solubilizing agents include non-ionic detergents such as n-octylglucoside, n-dodecylglucoside, n-octylmaltoside, octanoyl-N-methylglucamide, decanoyl-N-methylglucamide, Triton X-100, Triton X-114, Thesit, Isotridecypoly(ethylene glycol ether)n, 3-[(3-cholamidopropyl)dimethylammminio]-1-propane sulfonate (CHAPS), 3-[(3-cholamidopropyl)dimethylammminio]-2-hydroxy-1-propane sulfonate (CHAPSO), or N-dodecyl=N,N-dimethyl-3-ammonio-1-propane sulfonate.

In more than one embodiment of the above assay methods of the present invention, it may be desirable to immobilize either the polypeptide of the invention or its target molecule to facilitate separation of complexed from uncomplexed forms of one or both of the proteins, as well as to accommodate automation of the assay. Binding of a test compound to the polypeptide, or interaction of the polypeptide with a target molecule in the presence and absence of a candidate compound, can be accomplished in any vessel suitable for containing the reactants. Examples of such vessels include microtitre plates, test tubes, and micro-centrifuge tubes. In one embodiment, a fusion protein can be provided which adds a domain that allows one or both of the proteins to be bound to a matrix. For example, glutathione-S-transferase fusion proteins or glutathione-S-transferase fusion proteins can be adsorbed onto glutathione sepharose beads (Sigma Chemical; St. Louis, MO) or glutathione derivatized microtitre plates, which are then combined with the test compound or the test compound and either the non-adsorbed target protein or A polypeptide of the invention, and the mixture incubated under conditions conducive to complex formation (*e.g.*, at physiological conditions for salt and pH). Following incubation,

the beads or microtitre plate wells are washed to remove any unbound components and complex formation is measured either directly or indirectly, for example, as described above. Alternatively, the complexes can be dissociated from the matrix, and the level of binding or activity of the polypeptide of the invention can be determined using standard techniques.

Other techniques for immobilizing proteins on matrices can also be used in the screening assays of the invention. For example, either the polypeptide of the invention or its target molecule can be immobilized utilizing conjugation of biotin and streptavidin. Biotinylated polypeptide of the invention or target molecules can be prepared from biotin-NHS (N-hydroxy-succinimide) using techniques well known in the art (e.g., biotinylation kit, Pierce Chemicals; Rockford, IL), and immobilized in the wells of streptavidin-coated 96 well plates (Pierce Chemical). Alternatively, antibodies reactive with the polypeptide of the invention or target molecules but which do not interfere with binding of the polypeptide of the invention to its target molecule can be derivatized to the wells of the plate, and unbound target or polypeptide of the invention trapped in the wells by antibody conjugation. Methods for detecting such complexes, in addition to those described above for the GST-immobilized complexes, include immunodetection of complexes using antibodies reactive with the polypeptide of the invention or target molecule, as well as enzyme-linked assays which rely on detecting an enzymatic activity associated with the polypeptide of the invention or target molecule.

In another embodiment, modulators of expression of a polypeptide of the invention are identified in a method in which a cell is contacted with a candidate compound and the expression of the selected mRNA or protein (i.e., the mRNA or protein corresponding to a polypeptide or nucleic acid of the invention) in the cell is determined. The level of expression of the selected mRNA or protein in the presence of the candidate compound is compared to the level of expression of the selected mRNA or protein in the absence of the candidate compound. The candidate compound can then be identified as a modulator of expression of the polypeptide of the invention based on this comparison. For example, when expression of the selected mRNA or protein is greater (statistically significantly greater) in the presence of the candidate compound than in its absence, the candidate compound is identified as a stimulator of the selected mRNA or protein expression. Alternatively, when expression of the selected mRNA or protein is less (statistically significantly less) in the presence of the candidate compound than in its absence, the candidate compound is identified as an inhibitor of the selected mRNA or protein expression.

The level of the selected mRNA or protein expression in the cells can be determined by methods described herein.

In yet another aspect of the invention, a polypeptide of the inventions can be used as Abait proteins@ in a two-hybrid assay or three hybrid assay (*see, e.g.*, U.S. Patent No. 5,283,317; Zervos et al. (1993) *Cell* 72:223-232; Madura et al. (1993) *J. Biol. Chem.* 268:12046-12054; Bartel et al. (1993) *Bio/Techniques* 14:920-924; Iwabuchi et al. (1993) *Oncogene* 8:1693-1696; and PCT Publication No. WO 94/10300) to identify other proteins that bind to or interact with the polypeptide of the invention and modulate activity of the polypeptide of the invention. Such binding proteins are also likely to be involved in the propagation of signals by the polypeptide of the inventions as, for example, upstream or downstream elements of a signaling pathway involving the polypeptide of the invention.

This invention further pertains to novel agents identified by the above-described screening assays and uses thereof for treatments as described herein.

B. Detection Assays

Portions or fragments of the cDNA sequences identified herein (and the corresponding complete gene sequences) can be used in numerous ways as polynucleotide reagents. For example, these sequences can be used to: (i) map their respective genes on a chromosome and, thus, locate gene regions associated with genetic disease; (ii) identify an individual from a minute biological sample (tissue typing); and (iii) aid in forensic identification of a biological sample. These applications are described in the subsections below.

1. Chromosome Mapping

Once the sequence (or a portion of the sequence) of a gene has been isolated, this sequence can be used to map the location of the gene on a chromosome. Accordingly, nucleic acid molecules described herein or fragments thereof, can be used to map the location of the corresponding genes on a chromosome. The mapping of the sequences to chromosomes is an important first step in correlating these sequences with genes associated with disease.

Briefly, genes can be mapped to chromosomes by preparing PCR primers (preferably 15-25 bp in length) from the sequence of a gene of the invention. Computer analysis of the sequence of a gene of the invention can be used to rapidly select primers that do not span more

than one exon in the genomic DNA, thus complicating the amplification process. These primers can then be used for PCR screening of somatic cell hybrids containing individual human chromosomes. Only those hybrids containing the human gene corresponding to the gene sequences will yield an amplified fragment. For a review of this technique, see D'Eustachio et al. ((1983) *Science* 220:919-924).

PCR mapping of somatic cell hybrids is a rapid procedure for assigning a particular sequence to a particular chromosome. Three or more sequences can be assigned per day using a single thermal cycler. Using the nucleic acid sequences of the invention to design oligonucleotide primers, sublocalization can be achieved with panels of fragments from specific chromosomes. Other mapping strategies which can similarly be used to map a gene to its chromosome include *in situ* hybridization (described in Fan et al. (1990) *Proc. Natl. Acad. Sci. USA* 87:6223-27), pre-screening with labeled flow-sorted chromosomes (CITE), and pre-selection by hybridization to chromosome specific cDNA libraries. Fluorescence *in situ* hybridization (FISH) of a DNA sequence to a metaphase chromosomal spread can further be used to provide a precise chromosomal location in one step. For a review of this technique, see Verma et al., (Human Chromosomes: A Manual of Basic Techniques (Pergamon Press, New York, 1988)).

Reagents for chromosome mapping can be used individually to mark a single chromosome or a single site on that chromosome, or panels of reagents can be used for marking multiple sites and/or multiple chromosomes. Reagents corresponding to noncoding regions of the genes actually are preferred for mapping purposes. Coding sequences are more likely to be conserved within gene families, thus increasing the chance of cross hybridizations during chromosomal mapping.

Once a sequence has been mapped to a precise chromosomal location, the physical position of the sequence on the chromosome can be correlated with genetic map data. (Such data are found, for example, in V. McKusick, Mendelian Inheritance in Man, available on-line through Johns Hopkins University Welch Medical Library). The relationship between genes and disease, mapped to the same chromosomal region, can then be identified through linkage analysis (co-inheritance of physically adjacent genes), described in, *e.g.*, Egeland et al. (1987) *Nature* 325:783-787.

50

Moreover, differences in the DNA sequences between individuals affected and unaffected with a disease associated with a gene of the invention can be determined. If a mutation is observed in some or all of the affected individuals but not in any unaffected individuals, then the mutation is likely to be the causative agent of the particular disease. Comparison of affected and unaffected individuals generally involves first looking for structural alterations in the chromosomes such as deletions or translocations that are visible from chromosome spreads or detectable using PCR based on that DNA sequence. Ultimately, complete sequencing of genes from several individuals can be performed to confirm the presence of a mutation and to distinguish mutations from polymorphisms.

Furthermore, the nucleic acid sequences disclosed herein can be used to perform searches against "mapping databases", e.g., BLAST-type search, such that the chromosome position of the gene is identified by sequence homology or identity with known sequence fragments which have been mapped to chromosomes.

A polypeptide and fragments and sequences thereof and antibodies specific thereto can be used to map the location of the gene encoding the polypeptide on a chromosome. This mapping can be carried out by specifically detecting the presence of the polypeptide in members of a panel of somatic cell hybrids between cells of a first species of animal from which the protein originates and cells from a second species of animal and then determining which somatic cell hybrid(s) expresses the polypeptide and noting the chromosome(s) from the first species of animal that it contains. For examples of this technique, see Pajunen *et al.* (1988*) Cytogenet. Cell Genet.* 47:37-41 and Van Keuren *et al.* (1986*) Hum. Genet.* 74:34-40. Alternatively, the presence of the polypeptide in the somatic cell hybrids can be determined by assaying an activity or property of the polypeptide, for example, enzymatic activity, as described in Bordelon-Riser *et al.* (1979*) Somatic Cell Genetics* 5:597-613 and Owerbach *et al.* (1978*) Proc. Natl. Acad. Sci. USA* 75:5640-5644.

2. Tissue Typing

The nucleic acid sequences of the present invention can also be used to identify individuals from minute biological samples. The United States military, for example, is considering the use of restriction fragment length polymorphism (RFLP) for identification of its personnel. In this technique, an individual's genomic DNA is digested with one or more

51

restriction enzymes, and probed on a Southern blot to yield unique bands for identification. This method does not suffer from the current limitations of ADog Tags@ which can be lost, switched, or stolen, making positive identification difficult. The sequences of the present invention are useful as additional DNA markers for RFLP (described in U.S. Patent 5,272,057).

Furthermore, the sequences of the present invention can be used to provide an alternative technique that determines the actual base-by-base DNA sequence of selected portions of an individual's genome. Thus, the nucleic acid sequences described herein can be used to prepare two PCR primers from the 5' and 3' ends of the sequences. These primers can then be used to amplify an individual's DNA and subsequently sequence it.

Panels of corresponding DNA sequences from individuals, prepared in this manner, can provide unique individual identifications, as each individual will have a unique set of such DNA sequences due to allelic differences. The sequences of the present invention can be used to obtain such identification sequences from individuals and from tissue. The nucleic acid sequences of the invention uniquely represent portions of the human genome. Allelic variation occurs to some degree in the coding regions of these sequences, and to a greater degree in the noncoding regions. It is estimated that allelic variation between individual humans occurs with a frequency at about once per each 500 bases. Each of the sequences described herein can, to some degree, be used as a standard against which DNA from an individual can be compared for identification purposes. Because greater numbers of polymorphisms occur in the noncoding regions, fewer sequences are necessary to differentiate individuals. The noncoding sequences of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:9, SEQ ID NO:11, or SEQ ID NO:13 can comfortably provide positive individual identification with a panel of perhaps 10 to 1,000 primers which each yield a noncoding amplified sequence of 100 bases. If predicted coding sequences, such as those in SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:9, SEQ ID NO:11, or SEQ ID NO:13 are used, a more appropriate number of primers for positive individual identification would be 500 to 2,000.

If a panel of reagents from the nucleic acid sequences described herein is used to generate a unique identification database for an individual, those same reagents can later be used to identify tissue from that individual. Using the unique identification database, positive identification of the individual, living or dead, can be made from extremely small tissue samples.

3. Use of Partial Gene Sequences in Forensic Biology

DNA-based identification techniques can also be used in forensic biology. Forensic biology is a scientific field employing genetic typing of biological evidence found at a crime scene as a means for positively identifying, for example, a perpetrator of a crime. To make such an identification, PCR technology can be used to amplify DNA sequences taken from very small biological samples such as tissues, *e.g.*, hair or skin, or body fluids, *e.g.*, blood, saliva, or semen found at a crime scene. The amplified sequence can then be compared to a standard, thereby allowing identification of the origin of the biological sample.

The sequences of the present invention can be used to provide polynucleotide reagents, *e.g.*, PCR primers, targeted to specific loci in the human genome, which can enhance the reliability of DNA-based forensic identifications by, for example, providing another Aidentification marker@ (*i.e.* another DNA sequence that is unique to a particular individual). As mentioned above, actual base sequence information can be used for identification as an accurate alternative to patterns formed by restriction enzyme generated fragments. Sequences targeted to noncoding regions are particularly appropriate for this use as greater numbers of polymorphisms occur in the noncoding regions, making it easier to differentiate individuals using this technique. Examples of polynucleotide reagents include the nucleic acid sequences of the invention or portions thereof, *e.g.*, fragments derived from noncoding regions having a length of at least 20 or 30 bases.

The nucleic acid sequences described herein can further be used to provide polynucleotide reagents, *e.g.*, labeled or labelable probes which can be used in, for example, an *in situ* hybridization technique, to identify a specific tissue, *e.g.*, brain tissue. This can be very useful in cases where a forensic pathologist is presented with a tissue of unknown origin. Panels of such probes can be used to identify tissue by species and/or by organ type.

C. Predictive Medicine

The present invention also pertains to the field of predictive medicine in which diagnostic assays, prognostic assays, pharmacogenomics, and monitoring clinical trials are used for prognostic (predictive) purposes to thereby treat an individual prophylactically. Accordingly, one aspect of the present invention relates to diagnostic assays for determining expression of a polypeptide or nucleic acid of the invention and/or activity of a polypeptide of the invention, in

the context of a biological sample (*e.g.*, blood, serum, cells, tissue) to thereby determine whether an individual is afflicted with a disease or disorder, or is at risk of developing a disorder, associated with aberrant expression or activity of a polypeptide of the invention, such as a proliferative disorder, *e.g.*, psoriasis or cancer, or an angiogenic disorder. The invention also provides for prognostic (or predictive) assays for determining whether an individual is at risk of developing a disorder associated with aberrant expression or activity of a polypeptide of the invention. For example, mutations in a gene of the invention can be assayed in a biological sample. Such assays can be used for prognostic or predictive purpose to thereby prophylactically treat an individual prior to the onset of a disorder characterized by or associated with aberrant expression or activity of a polypeptide of the invention.

Another aspect of the invention provides methods for expression of a nucleic acid or polypeptide of the invention or activity of a polypeptide of the invention in an individual to thereby select appropriate therapeutic or prophylactic agents for that individual (referred to herein as "pharmacogenomics"). Pharmacogenomics allows for the selection of agents (*e.g.*, drugs) for therapeutic or prophylactic treatment of an individual based on the genotype of the individual (*e.g.*, the genotype of the individual examined to determine the ability of the individual to respond to a particular agent).

Yet another aspect of the invention pertains to monitoring the influence of agents (*e.g.*, drugs or other compounds) on the expression or activity of a polypeptide of the invention in clinical trials. These and other agents are described in further detail in the following sections.

1. Diagnostic Assays

An exemplary method for detecting the presence or absence of a polypeptide or nucleic acid of the invention in a biological sample involves obtaining a biological sample from a test subject and contacting the biological sample with a compound or an agent capable of detecting a polypeptide or nucleic acid (*e.g.*, mRNA, genomic DNA) of the invention such that the presence of a polypeptide or nucleic acid of the invention is detected in the biological sample. A preferred agent for detecting mRNA or genomic DNA encoding a polypeptide of the invention is a labeled nucleic acid probe capable of hybridizing to mRNA or genomic DNA encoding a polypeptide of the invention. The nucleic acid probe can be, for example, a full-length cDNA, such as the nucleic acid of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:9, SEQ ID NO:11, or

SEQ ID NO:13 or a portion thereof, such as an oligonucleotide of at least 15, 30, 50, 100, 250 or 500 contiguous nucleotides in length and sufficient to specifically hybridize under stringent conditions to a mRNA or genomic DNA encoding a polypeptide of the invention. Other suitable probes for use in the diagnostic assays of the invention are described herein.

A preferred agent for detecting a polypeptide of the invention is an antibody capable of binding to a polypeptide of the invention, preferably an antibody with a detectable label. Antibodies can be polyclonal, or more preferably, monoclonal. An intact antibody, or a fragment thereof (e.g., Fab or F(ab')$_2$) can be used. The term Alabeled@, with regard to the probe or antibody, is intended to encompass direct labeling of the probe or antibody by coupling (i.e., physically linking) a detectable substance to the probe or antibody, as well as indirect labeling of the probe or antibody by reactivity with another reagent that is directly labeled. Examples of indirect labeling include detection of a primary antibody using a fluorescently labeled secondary antibody and end-labeling of a DNA probe with biotin such that it can be detected with fluorescently labeled streptavidin. The term Abiological sample@ is intended to include tissues, cells and biological fluids isolated from a subject, as well as tissues, cells and fluids present within a subject. That is, the detection method of the invention can be used to detect mRNA, protein, or genomic DNA in a biological sample in vitro as well as in vivo. For example, in vitro techniques for detection of mRNA include Northern hybridizations and in situ hybridizations. In vitro techniques for detection of a polypeptide of the invention include enzyme linked immunosorbent assays (ELISAs), Western blots, immunoprecipitations and immunofluorescence. In vitro techniques for detection of genomic DNA include Southern hybridizations. Furthermore, in vivo techniques for detection of a polypeptide of the invention include introducing into a subject a labeled antibody directed against the polypeptide. For example, the antibody can be labeled with a radioactive marker whose presence and location in a subject can be detected by standard imaging techniques.

In one embodiment, the biological sample contains protein molecules from the test subject. Alternatively, the biological sample can contain mRNA molecules from the test subject or genomic DNA molecules from the test subject. A preferred biological sample is a peripheral blood leukocyte sample isolated by conventional means from a subject.

In another embodiment, the methods further involve obtaining a control biological sample from a control subject, contacting the control sample with a compound or agent capable

of detecting a polypeptide of the invention or mRNA or genomic DNA encoding a polypeptide of the invention, such that the presence of the polypeptide or mRNA or genomic DNA encoding the polypeptide is detected in the biological sample, and comparing the presence of the polypeptide or mRNA or genomic DNA encoding the polypeptide in the control sample with the presence of the polypeptide or mRNA or genomic DNA encoding the polypeptide in the test sample.

The invention also encompasses kits for detecting the presence of a polypeptide or nucleic acid of the invention in a biological sample (a test sample). Such kits can be used to determine if a subject is suffering from or is at increased risk of developing a disorder associated with aberrant expression of a Tango-71, Tango-79, or Tango-81 gene as discussed, for example, in sections above relating to uses of the sequences of the invention.

In another example, kits can be used to determine if a subject is suffering from or is at risk for disorders involving Tango-71, Tango-79, or Tango-81.

In another example, kits can be used to determine if a subject is suffering from or is at risk for which are associated with aberrant Tango-71, Tango-79, or Tango-81 family member activity and/or expression.

The kit, for example, can comprise a labeled compound or agent capable of detecting the polypeptide or mRNA encoding the polypeptide in a biological sample and means for determining the amount of the polypeptide or mRNA in the sample (*e.g.*, an antibody which binds the polypeptide or an oligonucleotide probe which binds to DNA or mRNA encoding the polypeptide). Kits can also include instructions for observing that the tested subject is suffering from or is at risk of developing a disorder associated with aberrant expression of the polypeptide if the amount of the polypeptide or mRNA encoding the polypeptide is above or below a normal level.

For antibody-based kits, the kit can comprise, for example: (1) a first antibody (*e.g.*, attached to a solid support) which binds to a polypeptide of the invention; and, optionally, (2) a second, different antibody which binds to either the polypeptide or the first antibody and is conjugated to a detectable agent.

For oligonucleotide-based kits, the kit can comprise, for example: (1) an oligonucleotide, *e.g.*, a detectably labeled oligonucleotide, which hybridizes to a nucleic acid sequence encoding a polypeptide of the invention or (2) a pair of primers useful for amplifying a nucleic acid

molecule encoding a polypeptide of the invention. The kit can also comprise, *e.g.*, a buffering agent, a preservative, or a protein stabilizing agent. The kit can also comprise components necessary for detecting the detectable agent (*e.g.*, an enzyme or a substrate). The kit can also contain a control sample or a series of control samples which can be assayed and compared to the test sample contained. Each component of the kit is usually enclosed within an individual container and all of the various containers are within a single package along with instructions for observing whether the tested subject is suffering from or is at risk of developing a disorder associated with aberrant expression of the polypeptide.

2. Prognostic Assays

The methods described herein can furthermore be utilized as diagnostic or prognostic assays to identify subjects having or at risk of developing a disease or disorder associated with aberrant expression or activity of a polypeptide of the invention. For example, the assays described herein, such as the preceding diagnostic assays or the following assays, can be utilized to identify a subject having or at risk of developing a disorder associated with aberrant expression or activity of a polypeptide of the invention, *e.g.*, an immunologic disorder, or embryonic disorders. Alternatively, the prognostic assays can be utilized to identify a subject having or at risk for developing such a disease or disorder. Thus, the present invention provides a method in which a test sample is obtained from a subject and a polypeptide or nucleic acid (*e.g.*, mRNA, genomic DNA) of the invention is detected, wherein the presence of the polypeptide or nucleic acid is diagnostic for a subject having or at risk of developing a disease or disorder associated with aberrant expression or activity of the polypeptide. As used herein, a "test sample" refers to a biological sample obtained from a subject of interest. For example, a test sample can be a biological fluid (*e.g.*, serum), cell sample, or tissue.

The prognostic assays described herein, for example, can be used to identify a subject having or at risk of developing disorders such as disorders discussed, for example, in sections above relating to uses of the sequences of the invention. For example, prognostic assays described herein can be used to identify a subject having or at risk of developing immunological disorders, *e.g.*, autoimmune disorders (*e.g.*, arthritis, graft rejection (*e.g.*, allograft rejection), T cell disorders (*e.g.*, AIDS)), inflammatory disorders (*e.g.*, bacterial infection, psoriasis, septicemia, cerebral malaria, inflammatory bowel disease, arthritis (*e.g.*, rheumatoid arthritis,

osteoarthritis)), and allergic inflammatory disorders (*e.g.*, asthma, psoriasis), which are associated with aberrant Tango-71, Tango-79, or Tango-81 activity and/or expression.

In another example, prognostic assays described herein can be used to identify a subject having or at risk of developing brain-related disorders, inflammations (*e.g.*, bacterial and viral meningitis, encephalitis, and cerebral toxoplasmosis), and tumors (*e.g.*, astrocytoma), and to treat injury or trauma to the brain, which are associated with aberrant Tango-71, Tango-79, or Tango-81 family member activity and/or expression.

Furthermore, the prognostic assays described herein can be used to determine whether a subject can be administered an agent (*e.g.*, an agonist, antagonist, peptidomimetic, protein, peptide, nucleic acid, small molecule, or other drug candidate) to treat a disease or disorder associated with aberrant expression or activity of a polypeptide of the invention. For example, such methods can be used to determine whether a subject can be effectively treated with a specific agent or class of agents (*e.g.*, agents of a type which decrease activity of the polypeptide). Thus, the present invention provides methods for determining whether a subject can be effectively treated with an agent for a disorder associated with aberrant expression or activity of a polypeptide of the invention in which a test sample is obtained and the polypeptide or nucleic acid encoding the polypeptide is detected (*e.g.*, wherein the presence of the polypeptide or nucleic acid is diagnostic for a subject that can be administered the agent to treat a disorder associated with aberrant expression or activity of the polypeptide).

The methods of the invention can also be used to detect genetic lesions or mutations in a gene of the invention, thereby determining if a subject with the lesioned gene is at risk for a disorder characterized aberrant expression or activity of a polypeptide of the invention. In preferred embodiments, the methods include detecting, in a sample of cells from the subject, the presence or absence of a genetic lesion or mutation characterized by at least one of an alteration affecting the integrity of a gene encoding the polypeptide of the invention, or the mis-expression of the gene encoding the polypeptide of the invention. For example, such genetic lesions or mutations can be detected by ascertaining the existence of at least one of: 1) a deletion of one or more nucleotides from the gene; 2) an addition of one or more nucleotides to the gene; 3) a substitution of one or more nucleotides of the gene; 4) a chromosomal rearrangement of the gene; 5) an alteration in the level of a messenger RNA transcript of the gene; 6) an aberrant modification of the gene, such as of the methylation pattern of the genomic DNA; 7) the

presence of a non-wild type splicing pattern of a messenger RNA transcript of the gene; 8) a non-wild type level of a the protein encoded by the gene; 9) an allelic loss of the gene; and 10) an inappropriate post-translational modification of the protein encoded by the gene. As described herein, there are a large number of assay techniques known in the art that can be used for detecting lesions in a gene.

In certain embodiments, detection of the lesion involves the use of a probe/primer in a polymerase chain reaction (PCR) (see, e.g., U.S. Patent Nos. 4,683,195 and 4,683,202), such as anchor PCR or RACE PCR, or, alternatively, in a ligation chain reaction (LCR) (see, e.g., Landegran et al. (1988) Science 241:1077-1080; and Nakazawa et al. (1994) Proc. Natl. Acad. Sci. USA 91:360-364), the latter of which can be particularly useful for detecting point mutations in a gene (see, e.g., Abravaya et al. (1995) Nucleic Acids Res. 23:675-682). This method can include the steps of collecting a sample of cells from a patient, isolating nucleic acid (e.g., genomic, mRNA or both) from the cells of the sample, contacting the nucleic acid sample with one or more primers which specifically hybridize to the selected gene under conditions such that hybridization and amplification of the gene (if present) occurs, and detecting the presence or absence of an amplification product, or detecting the size of the amplification product and comparing the length to a control sample. It is anticipated that PCR and/or LCR may be desirable to use as a preliminary amplification step in conjunction with any of the techniques used for detecting mutations described herein.

Alternative amplification methods include: self sustained sequence replication (Guatelli et al. (1990) Proc. Natl. Acad. Sci. USA 87:1874-1878), transcriptional amplification system (Kwoh, et al. (1989) Proc. Natl. Acad. Sci. USA 86:1173-1177), Q-Beta Replicase (Lizardi et al. (1988) Bio/Technology 6:1197), or any other nucleic acid amplification method, followed by the detection of the amplified molecules using techniques well known to those of skill in the art. These detection schemes are especially useful for the detection of nucleic acid molecules if such molecules are present in very low numbers.

In an alternative embodiment, mutations in a selected gene from a sample cell can be identified by alterations in restriction enzyme cleavage patterns. For example, sample and control DNA is isolated, amplified (optionally), digested with one or more restriction endonucleases, and fragment length sizes are determined by gel electrophoresis and compared. Differences in fragment length sizes between sample and control DNA indicates mutations in the

sample DNA. Moreover, the use of sequence specific ribozymes (*see, e.g.,* U.S. Patent No. 5,498,531) can be used to score for the presence of specific mutations by development or loss of a ribozyme cleavage site.

In other embodiments genetic mutations can be identified by hybridizing a sample and control nucleic acids, *e.g.,* DNA or RNA, to high density arrays containing hundreds or thousands of oligonucleotides probes (Cronin et al. (1996) *Human Mutation* 7:244-255; Kozal et al. (1996) *Nature Medicine* 2:753-759). For example, genetic mutations can be identified in two-dimensional arrays containing light-generated DNA probes as described in Cronin et al., *supra.* Briefly, a first hybridization array of probes can be used to scan through long stretches of DNA in a sample and control to identify base changes between the sequences by making linear arrays of sequential overlapping probes. This step allows the identification of point mutations. This step is followed by a second hybridization array that allows the characterization of specific mutations by using smaller, specialized probe arrays complementary to all variants or mutations detected. Each mutation array is composed of parallel probe sets, one complementary to the wild-type gene and the other complementary to the mutant gene.

In yet another embodiment, any of a variety of sequencing reactions known in the art can be used to directly sequence the selected gene and detect mutations by comparing the sequence of the sample nucleic acids with the corresponding wild-type (control) sequence. Examples of sequencing reactions include those based on techniques developed by Maxim and Gilbert ((1977) *Proc. Natl. Acad. Sci. USA* 74:560) or Sanger ((1977) *Proc. Natl. Acad. Sci. USA* 74:5463). It is also contemplated that any of a variety of automated sequencing procedures can be utilized when performing the diagnostic assays ((1995) *Bio/Techniques* 19:448), including sequencing by mass spectrometry *(see, e.g.,* PCT Publication No. WO 94/16101; Cohen et al. (1996) *Adv. Chromatogr.* 36:127-162; and Griffin et al. (1993) *Appl. Biochem. Biotechnol.* 38:147-159).

Other methods for detecting mutations in a selected gene include methods in which protection from cleavage agents is used to detect mismatched bases in RNA/RNA or RNA/DNA heteroduplexes (Myers et al. (1985) *Science* 230:1242). In general, the technique of Amismatch cleavage@ entails providing heteroduplexes formed by hybridizing (labeled) RNA or DNA containing the wild-type sequence with potentially mutant RNA or DNA obtained from a tissue sample. The double-stranded duplexes are treated with an agent which cleaves single-stranded regions of the duplex such as which will exist due to basepair mismatches between the control

and sample strands. RNA/DNA duplexes can be treated with RNase to digest mismatched regions, and DNA/DNA hybrids can be treated with S1 nuclease to digest mismatched regions.

In other embodiments, either DNA/DNA or RNA/DNA duplexes can be treated with hydroxylamine or osmium tetroxide and with piperidine in order to digest mismatched regions. After digestion of the mismatched regions, the resulting material is then separated by size on denaturing polyacrylamide gels to determine the site of mutation. *See, e.g.*, Cotton et al. (1988) *Proc. Natl. Acad. Sci. USA* 85:4397; Saleeba et al. (1992) *Methods Enzymol.* 217:286-295. In a preferred embodiment, the control DNA or RNA can be labeled for detection.

In still another embodiment, the mismatch cleavage reaction employs one or more proteins that recognize mismatched base pairs in double-stranded DNA (so called ADNA mismatch repair@ enzymes) in defined systems for detecting and mapping point mutations in cDNAs obtained from samples of cells. For example, the mutY enzyme of E. coli cleaves A at G/A mismatches and the thymidine DNA glycosylase from HeLa cells cleaves T at G/T mismatches (Hsu et al. (1994) *Carcinogenesis* 15:1657-1662). According to an exemplary embodiment, a probe based on a selected sequence, *e.g.*, a wild-type sequence, is hybridized to a cDNA or other DNA product from a test cell(s). The duplex is treated with a DNA mismatch repair enzyme, and the cleavage products, if any, can be detected from electrophoresis protocols or the like. *See, e.g.*, U.S. Patent No. 5,459,039.

In other embodiments, alterations in electrophoretic mobility will be used to identify mutations in genes. For example, single strand conformation polymorphism (SSCP) may be used to detect differences in electrophoretic mobility between mutant and wild type nucleic acids (Orita et al. (1989) *Proc. Natl. Acad. Sci. USA* 86:2766; *see also* Cotton (1993) *Mutat. Res.* 285:125-144; Hayashi (1992) *Genet. Anal. Tech. Appl.* 9:73-79). Single-stranded DNA fragments of sample and control nucleic acids will be denatured and allowed to renature. The secondary structure of single-stranded nucleic acids varies according to sequence, and the resulting alteration in electrophoretic mobility enables the detection of even a single base change. The DNA fragments may be labeled or detected with labeled probes. The sensitivity of the assay may be enhanced by using RNA (rather than DNA), in which the secondary structure is more sensitive to a change in sequence. In a preferred embodiment, the subject method utilizes heteroduplex analysis to separate double stranded heteroduplex molecules on the basis of changes in electrophoretic mobility (Keen et al. (1991) *Trends Genet.* 7:5).

In yet another embodiment, the movement of mutant or wild-type fragments in polyacrylamide gels containing a gradient of denaturant is assayed using denaturing gradient gel electrophoresis (DGGE) (Myers et al. (1985) *Nature* 313:495). When DGGE is used as the method of analysis, DNA will be modified to insure that it does not completely denature, for example by adding a `GC clamp of approximately 40 bp of high-melting GC-rich DNA by PCR. In a further embodiment, a temperature gradient is used in place of a denaturing gradient to identify differences in the mobility of control and sample DNA (Rosenbaum and Reissner (1987) *Biophys. Chem.* 265:12753).

Examples of other techniques for detecting point mutations include, but are not limited to, selective oligonucleotide hybridization, selective amplification, or selective primer extension. For example, oligonucleotide primers may be prepared in which the known mutation is placed centrally and then hybridized to target DNA under conditions which permit hybridization only if a perfect match is found (Saiki et al. (1986) *Nature* 324:163); Saiki et al. (1989) *Proc. Natl. Acad. Sci. USA* 86:6230). Such allele specific oligonucleotides are hybridized to PCR amplified target DNA or a number of different mutations when the oligonucleotides are attached to the hybridizing membrane and hybridized with labeled target DNA.

Alternatively, allele specific amplification technology that depends on selective PCR amplification may be used in conjunction with the instant invention. Oligonucleotides used as primers for specific amplification may carry the mutation of interest in the center of the molecule (so that amplification depends on differential hybridization) (Gibbs et al. (1989) *Nucleic Acids Res.* 17:2437-2448) or at the extreme 3' end of one primer where, under appropriate conditions, mismatch can prevent or reduce polymerase extension (Prossner (1993) *Tibtech* 11:238). In addition, it may be desirable to introduce a novel restriction site in the region of the mutation to create cleavage-based detection (Gasparini et al. (1992) *Mol. Cell Probes* 6:1). It is anticipated that in certain embodiments amplification may also be performed using Taq ligase for amplification (Barany (1991) *Proc. Natl. Acad. Sci. USA* 88:189). In such cases, ligation will occur only if there is a perfect match at the 3' end of the 5' sequence making it possible to detect the presence of a known mutation at a specific site by looking for the presence or absence of amplification.

The methods described herein may be performed, for example, by utilizing pre-packaged diagnostic kits comprising at least one probe nucleic acid or antibody reagent described herein,

62

which may be conveniently used, *e.g.*, in clinical settings to diagnose patients exhibiting symptoms or family history of a disease or illness involving a gene encoding a polypeptide of the invention. Furthermore, any cell type or tissue, *e.g.*, preferably peripheral blood leukocytes, in which the polypeptide of the invention is expressed may be utilized in the prognostic assays described herein.

3. Pharmacogenomics

Agents or modulators that have a stimulatory or inhibitory effect on activity or expression of a polypeptide of the invention as identified by a screening assay described herein can be administered to individuals to treat (prophylactically or therapeutically) disorders associated with aberrant activity of the polypeptide. In conjunction with such treatment, the pharmacogenomics (*i.e.*, the study of the relationship between an individual's genotype and that individual's response to a foreign compound or drug) of the individual may be considered. Differences in metabolism of therapeutics can lead to severe toxicity or therapeutic failure by altering the relation between dose and blood concentration of the pharmacologically active drug. Thus, the pharmacogenomics of the individual permits the selection of effective agents (*e.g.*, drugs) for prophylactic or therapeutic treatments based on a consideration of the individual's genotype. Such pharmacogenomics can further be used to determine appropriate dosages and therapeutic regimens. Accordingly, the activity of a polypeptide of the invention, expression of a nucleic acid of the invention, or mutation content of a gene of the invention in an individual can be determined to thereby select appropriate agent(s) for therapeutic or prophylactic treatment of the individual.

Pharmacogenomics deals with clinically significant hereditary variations in the response to drugs due to altered drug disposition and abnormal action in affected persons. *See, e.g.*, Linder (1997) *Clin. Chem.* 43(2):254-266. In general, two types of pharmacogenetic conditions can be differentiated. Genetic conditions transmitted as a single factor altering the way drugs act on the body are referred to as Aaltered drug action.@ Genetic conditions transmitted as single factors altering the way the body acts on drugs are referred to as Aaltered drug metabolism@. These pharmacogenetic conditions can occur either as rare defects or as polymorphisms. For example, glucose-6-phosphate dehydrogenase deficiency (G6PD) is a common inherited

enzymopathy in which the main clinical complication is haemolysis after ingestion of oxidant drugs (anti-malarials, sulfonamides, analgesics, nitrofurans) and consumption of fava beans.

As an illustrative embodiment, the activity of drug metabolizing enzymes is a major determinant of both the intensity and duration of drug action. The discovery of genetic polymorphisms of drug metabolizing enzymes (e.g., N-acetyltransferase 2 (NAT 2) and cytochrome P450 enzymes CYP2D6 and CYP2C19) has provided an explanation as to why some patients do not obtain the expected drug effects or show exaggerated drug response and serious toxicity after taking the standard and safe dose of a drug. These polymorphisms are expressed in two phenotypes in the population, the extensive metabolizer (EM) and poor metabolizer (PM). The prevalence of PM is different among different populations. For example, the gene coding for CYP2D6 is highly polymorphic and several mutations have been identified in PM, which all lead to the absence of functional CYP2D6. Poor metabolizers of CYP2D6 and CYP2C19 quite frequently experience exaggerated drug response and side effects when they receive standard doses. If a metabolite is the active therapeutic moiety, a PM will show no therapeutic response, as demonstrated for the analgesic effect of codeine mediated by its CYP2D6-formed metabolite morphine. The other extreme are the so called ultra-rapid metabolizers who do not respond to standard doses. Recently, the molecular basis of ultra-rapid metabolism has been identified to be due to CYP2D6 gene amplification.

Thus, the activity of a polypeptide of the invention, expression of a nucleic acid encoding the polypeptide, or mutation content of a gene encoding the polypeptide in an individual can be determined to thereby select appropriate agent(s) for therapeutic or prophylactic treatment of the individual. In addition, pharmacogenetic studies can be used to apply genotyping of polymorphic alleles encoding drug-metabolizing enzymes to the identification of an individual's drug responsiveness phenotype. This knowledge, when applied to dosing or drug selection, can avoid adverse reactions or therapeutic failure and thus enhance therapeutic or prophylactic efficiency when treating a subject with a modulator of activity or expression of the polypeptide, such as a modulator identified by one of the exemplary screening assays described herein.

4. Monitoring of Effects During Clinical Trials

Monitoring the influence of agents (e.g., drugs, compounds) on the expression or activity of a polypeptide of the invention (e.g., the ability to modulate aberrant cell proliferation

64

chemotaxis, and/or differentiation) can be applied not only in basic drug screening, but also in clinical trials. For example, the effectiveness of an agent, as determined by a screening assay as described herein, to increase gene expression, protein levels or protein activity, can be monitored in clinical trials of subjects exhibiting decreased gene expression, protein levels, or protein activity. Alternatively, the effectiveness of an agent, as determined by a screening assay, to decrease gene expression, protein levels or protein activity, can be monitored in clinical trials of subjects exhibiting increased gene expression, protein levels, or protein activity. In such clinical trials, expression or activity of a polypeptide of the invention and preferably, that of other polypeptide that have been implicated in for example, a cellular proliferation disorder, can be used as a marker of the immune responsiveness of a particular cell.

For example, and not by way of limitation, genes, including those of the invention, that are modulated in cells by treatment with an agent (*e.g.*, compound, drug or small molecule) that modulates activity or expression of a polypeptide of the invention (*e.g.*, as identified in a screening assay described herein) can be identified. Thus, to study the effect of agents on cellular proliferation disorders, for example, in a clinical trial, cells can be isolated and RNA prepared and analyzed for the levels of expression of a gene of the invention and other genes implicated in the disorder. The levels of gene expression (*i.e.*, a gene expression pattern) can be quantified by Northern blot analysis or RT-PCR, as described herein, or alternatively by measuring the amount of protein produced, by one of the methods as described herein, or by measuring the levels of activity of a gene of the invention or other genes. In this way, the gene expression pattern can serve as a marker, indicative of the physiological response of the cells to the agent. Accordingly, this response state may be determined before, and at various points during, treatment of the individual with the agent.

In a preferred embodiment, the present invention provides a method for monitoring the effectiveness of treatment of a subject with an agent (*e.g.*, an agonist, antagonist, peptidomimetic, protein, peptide, nucleic acid, small molecule, or other drug candidate identified by the screening assays described herein) comprising the steps of (i) obtaining a pre-administration sample from a subject prior to administration of the agent; (ii) detecting the level of the polypeptide or nucleic acid of the invention in the preadministration sample; (iii) obtaining one or more post-administration samples from the subject; (iv) detecting the level the of the polypeptide or nucleic acid of the invention in the post-administration samples; (v)

comparing the level of the polypeptide or nucleic acid of the invention in the pre-administration sample with the level of the polypeptide or nucleic acid of the invention in the post-administration sample or samples; and (vi) altering the administration of the agent to the subject accordingly. For example, increased administration of the agent may be desirable to increase the expression or activity of the polypeptide to higher levels than detected, *i.e.*, to increase the effectiveness of the agent. Alternatively, decreased administration of the agent may be desirable to decrease expression or activity of the polypeptide to lower levels than detected, *i.e.*, to decrease the effectiveness of the agent.

C. Methods of Treatment

Tango-71, Tango-79, and Tango-81 polypeptides, nucleic acids, and modulators thereof can be used to modulate the function, morphology, proliferation and/or differentiation of cells in the tissues in which it is expressed. Such molecules can be used to treat disorders associated with abnormal or aberrant metabolism or function of cells in the tissues in which it is expressed. Tissues in which nucleic acids and polypeptides of the invention are expressed include, for example, pancreas, kidney, testis, heart, brain, liver, placenta, lung, skeletal muscle, or small intestine.

As revealed by Northern blot analysis, Tango-71, Tango-79, and Tango-81 are expressed in the brain. Consequently, Tango-71, Tango-79, and Tango-81 polypeptides, nucleic acids, and modulators thereof can be used to treat disorders of the brain, such as cerebral edema, hydrocephalus, brain herniations, iatrogenic disease (due to, e.g., infection, toxins, or drugs), inflammations (e.g., bacterial and viral meningitis, encephalitis, and cerebral toxoplasmosis), cerebrovascular diseases (e.g., hypoxia, ischemia, and infarction, intracranial hemorrhage and vascular malformations, and hypertensive encephalopathy), and tumors (e.g., neuroglial tumors, neuronal tumors, tumors of pineal cells, meningeal tumors, primary and secondary lymphomas, intracranial tumors, and medulloblastoma), and to treat injury or trauma to the brain.

As revealed by *in situ* hybridization, Tango-71 and Tango-79 are expressed in the eye and Harderian gland. Consequently, Tango-71 and Tango-79 polypeptides, nucleic acids, and modulators thereof can be used to treat eye disorders, e.g., Retinitis Pigmentosa, Cataract, Color Blindness, Conjunctivitis, Dry Eyes, Glaucoma, Keratoconus, Macular Degeneration, Microphthalmia and Anophthalmia, Myopia, Nystagmus, Retinitis Pigmentosa, and Trachoma.

As revealed by Northern blot analysis, Tango-71 and Tango-81 are expressed in the cardiovascular system. Consequently, Tango-71 and Tango-81 polypeptides, nucleic acids, and modulators thereof can be used to treat cardiovascular disorders, such as ischemic heart disease (e.g., angina pectoris, myocardial infarction, and chronic ischemic heart disease), hypertensive heart disease, pulmonary heart disease, valvular heart disease (e.g., rheumatic fever and rheumatic heart disease, endocarditis, mitral valve prolapse, and aortic valve stenosis), congenital heart disease (e.g., valvular and vascular obstructive lesions, atrial or ventricular septal defect, and patent ductus arteriosus), or myocardial disease (e.g., myocarditis, congestive cardiomyopathy, and hypertrophic cariomyopathy).

As revealed by Northern blot analysis, Tango-71 and Tango-81 are expressed in the heart. Consequently, Tango-71 and Tango-81 nucleic acids, proteins, and modulators thereof can be used to treat heart disorders, e.g., ischemic heart disease, atherosclerosis, hypertension, angina pectoris, Hypertrophic Cardiomyopathy, and congenital heart disease.

As revealed by Northern blot analysis, Tango-71 and Tango-81 are expressed in the spleen. In situ hybridization analysis revealed that Tango-79 is also expressed in the spleen. Consequently, Tango-71, Tango-79, and Tango-81 nucleic acids, proteins, and modulators thereof can be used to modulate the proliferation, differentiation, and/or function of cells that form the spleen, e.g., cells of the splenic connective tissue, e.g., splenic smooth muscle cells and/or endothelial cells of the splenic blood vessels. Tango-71, Tango-79, and Tango-81 nucleic acids, proteins, and modulators thereof can also be used to modulate the proliferation, differentiation, and/or function of cells that are processed, e.g., regenerated or phagocytized within the spleen, e.g., erythrocytes and/or B and T lymphocytes and macrophages. Thus, Tango-71, Tango-79, and Tango-81 nucleic acids, proteins, and modulators thereof can be used to treat spleen, e.g., the fetal spleen, associated diseases and disorders. Examples of splenic diseases and disorders include e.g., splenic lymphoma and/or splenomegaly, and/or phagocytotic disorders, e.g., those inhibiting macrophage engulfment of bacteria and viruses in the bloodstream.

As revealed by Northern blot analysis, Tango-71 and Tango-81 are expressed in the lung. Consequently, Tango-71 and Tango-81 polypeptides, nucleic acids, and modulators thereof can be used to treat pulmonary (lung) disorders, such as atelectasis, cystic fibrosis, rheumatoid lung disease, pulmonary congestion or edema, chronic obstructive airway disease (e.g., emphysema,

67

1855,2058-001

chronic bronchitis, bronchial asthma, and bronchiectasis), diffuse interstitial diseases (e.g., sarcoidosis, pneumoconiosis, hypersensitivity pneumonitis, bronchiolitis, Goodpasture's syndrome, idiopathic pulmonary fibrosis, idiopathic pulmonary hemosiderosis, pulmonary alveolar proteinosis, desquamative interstitial pneumonitis, chronic interstitial pneumonia, fibrosing alveolitis, hamman-rich syndrome, pulmonary eosinophilia, diffuse interstitial fibrosis, Wegener's granulomatosis, lymphomatoid granulomatosis, and lipid pneumonia), or tumors (e.g., bronchogenic carcinoma, bronchiolovlveolar carcinoma, bronchial carcinoid, hamartoma, and mesenchymal tumors).

As revealed by Northern blot analysis, Tango-71 is expressed in the pancreas. Consequently, Tango-71 polypeptides, nucleic acids, and modulators thereof can be used to treat pancreatic disorders, such as pancreatitis (e.g., acute hemorrhagic pancreatitis and chronic pancreatitis), pancreatic cysts (e.g., congenital cysts, pseudocysts, and benign or malignant neoplastic cysts), pancreatic tumors (e.g., pancreatic carcinoma and adenoma), diabetes mellitus (e.g., insulin- and non-insulin-dependent types, impaired glucose tolerance, and gestational diabetes), or islet cell tumors (e.g., insulinomas, adenomas, Zollinger-Ellison syndrome, glucagonomas, and somatostatinoma).

As revealed by Northern blot analysis, Tango-71 and Tango-81 are expressed in the liver. Consequently, Tango-71 and Tango-81 polypeptides, nucleic acids, and modulators thereof can be used to treat hepatic (liver) disorders, such as jaundice, hepatic failure, hereditary hyperbiliruinemias (e.g., Gilbert's syndrome, Crigler-Naijar syndromes and Dubin-Johnson and Rotor's syndromes), hepatic circulatory disorders (e.g., hepatic vein thrombosis and portal vein obstruction and thrombosis), hepatitis (e.g., chronic active hepatitis, acute viral hepatitis, and toxic and drug-induced hepatitis), cirrhosis (e.g., alcoholic cirrhosis, biliary cirrhosis, and hemochromatosis), or malignant tumors (e.g., primary carcinoma, hepatoma, hepatoblastoma, liver cysts, and angiosarcoma).

As revealed by Northern blot analysis, Tango-71 and Tango-81 are expressed in the kidney. Consequently, Tango-71 and Tango-81 polypeptides, nucleic acids, and modulators thereof can be used to treat renal (kidney) disorders, such as glomerular diseases (e.g., acute and chronic glomerulonephritis, rapidly progressive glomerulonephritis, nephrotic syndrome, focal proliferative glomerulonephritis, glomerular lesions associated with systemic disease, such as systemic lupus erythematosus, Goodpasture's syndrome, multiple myeloma, diabetes, polycystic

kidney disease, neoplasia, sickle cell disease, and chronic inflammatory diseases), tubular diseases (e.g., acute tubular necrosis and acute renal failure, polycystic renal diseasemedullary sponge kidney, medullary cystic disease, nephrogenic diabetes, and renal tubular acidosis), tubulointerstitial diseases (e.g., pyelonephritis, drug and toxin induced tubulointerstitial nephritis, hypercalcemic nephropathy, and hypokalemic nephropathy) acute and rapidly progressive renal failure, chronic renal failure, nephrolithiasis, gout, vascular diseases (e.g., hypertension and nephrosclerosis, microangiopathic hemolytic anemia, atheroembolic renal disease, diffuse cortical necrosis, and renal infarcts), or tumors (e.g., renal cell carcinoma and nephroblastoma).

As revealed by Northern blot analysis, Tango-71 and Tango-81 are expressed in the reproductive system. Consequently, Tango-71 and Tango-81 can be used to treat reproductive disorders, including ovulation disorder, blockage of the fallopian tubes (e.g., due to pelvic inflammatory disease or endometriosis), disorders due to infections (e.g., toxic shock syndrome, chlamydia infection, Herpes infection, human papillomavirus infection), and ovarian disorders (e.g., ovarian cyst, ovarian fibroma, ovarian endometriosis, ovarian teratoma).

As revealed by Northern blot analysis, Tango-71 is expressed in the ovaries. Consequently, Tango-71 polypeptides, nucleic acids, and modulators thereof can be used to treat ovarian disorders, such as ovarian endometriosis, non-neoplastic cysts (e.g., follicular and luteal cysts and polycystic ovaries) and tumors (e.g., tumors of surface epithelium, germ cell tumors, ovarian fibroma, sex cord-stromal tumors, and ovarian cancers (e.g., metastatic carcinomas, and ovarian teratoma).

As revealed by Northern blot analysis, Tango-71 is expressed in the placenta. Consequently, Tango-71 polypeptides, nucleic acids, and modulators thereof can be used to treat placental disorders, such as toxemia of pregnancy (e.g., preeclampsia and eclampsia), placentitis, or spontaneous abortion.

As revealed by Northern blot analysis, Tango-71 and Tango-81 are expressed in the testes. Consequently, Tango-71 and Tango-81 polypeptides, nucleic acids, and modulators thereof can be used to treat testicular disorders, such as unilateral testicular enlargement (e.g., nontuberculous, granulomatous orchitis); inflammatory diseases resulting in testicular dysfunction (e.g., gonorrhea and mumps); cryptorchidism; sperm cell disorders (e.g., immotile cilia syndrome and germinal cell aplasia); acquired testicular defects (e.g., viral orchitis); and

69

tumors (e.g., germ cell tumors, interstitial cell tumors, androblastoma, testicular lymphoma and adenomatoid tumors).

As revealed by Northern blot analysis, Tango-71 is expressed in the prostate. Consequently, Tango-71 polypeptides, nucleic acids, and modulators thereof can be used to treat prostate disorders, such as inflammatory diseases (e.g., acute and chronic prostatitis and granulomatous prostatitis), hyperplasia (e.g., benign prostatic hypertrophy or hyperplasia), or tumors (e.g., carcinomas).

As revealed by Northern blot analysis, Tango-71 is expressed in the intestines. Consequently, Tango-71 polypeptides, nucleic acids, and modulators thereof can be used to treat intestinal disorders, such as ischemic bowel disease, infective enterocolitis, Crohn's disease, benign tumors, malignant tumors (e.g., argentaffinomas, lymphomas, adenocarcinomas, and sarcomas), malabsorption syndromes (e.g., celiac disease, tropical sprue, Whipple's disease, and abetalipoproteinemia), obstructive lesions, hernias, intestinal adhesions, intussusception, or volvulus.

As revealed by Northern blot analysis, Tango-71 is expressed in the colon. Consequently, Tango-71 polypeptides, nucleic acids, and modulators thereof can be used to treat colonic disorders, such as congenital anomalies (e.g., megacolon and imperforate anus), idiopathic disorders (e.g., diverticular disease and melanosis coli), vascular lesions (e.g., ischemic colistis, hemorrhoids, angiodysplasia), inflammatory diseases (e.g., colitis (e.g., idiopathic ulcerative colitis, pseudomembranous colitis), and lymphopathia venereum), and tumors (e.g., hyperplastic polyps, adenomatous polyps, bronchogenic cancer, colonic carcinoma, squamous cell carcinoma, adenoacanthomas, sarcomas, lymphomas, argentaffinomas, carcinoids, and melanocarcinomas).

As revealed by Northern blot analysis, Tango-71 and Tango-81 are expressed in skeletal muscle tissue. Consequently, Tango-71 and Tango-81 polypeptides, nucleic acids, and modulators thereof can be used to treat disorders of skeletal muscle, such as muscular dystrophy (e.g., Duchenne Muscular Dystrophy, Becker Muscular Dystrophy, Emery-Dreifuss Muscular Dystrophy,Limb-Girdle Muscular Dystrophy, Facioscapulohumeral Muscular Dystrophy, Myotonic Dystrophy, Oculopharyngeal Muscular Dystrophy, Distal Muscular Dystrophy, and Congenital Muscular Dystrophy), motor neuron diseases (e.g., Amyotrophic Lateral Sclerosis, Infantile Progressive Spinal Muscular Atrophy, Intermediate Spinal Muscular Atrophy, Spinal Bulbar Muscular Atrophy, and Adult Spinal Muscular Atrophy), myopathies (e.g., inflammatory

myopathies (e.g., Dermatomyositis and Polymyositis), Myotonia Congenita, Paramyotonia Congenita, Central Core Disease, Nemaline Myopathy, Myotubular Myopathy, and Periodic Paralysis), and metabolic diseases of muscle (e.g., Phosphorylase Deficiency, Acid Maltase Deficiency, Phosphofructokinase Deficiency, Debrancher Enzyme Deficiency, Mitochondrial Myopathy, Carnitine Deficiency, Carnitine Palmityl Transferase Deficiency, Phosphoglycerate Kinase Deficiency, Phosphoglycerate Mutase Deficiency, Lactate Dehydrogenase Deficiency, and Myoadenylate Deaminase Deficiency).

The nucleic acids or polypeptides of the invention can be used to treat proliferative disorders, e.g., neoplasms or tumors (e.g., a carcinoma, a sarcoma, adenoma, or myeloid leukemia).

Disorders associated with abnormal Tango-71, Tango-79, or Tango-81 activity or expression may include proliferative disorders (e.g., carcinoma, lymphoma, e.g., follicular lymphoma).

Disorders associated with abnormal Tango-71 activity or expression may include inflammatory disorders (e.g., bacterial infection, psoriasis, septicemia, cerebral malaria, inflammatory bowel disease (e.g., ulcerative colitis, Crohn's disease), arthritis (e.g., rheumatoid arthritis, osteoarthritis), and allergic inflammatory disorders (e.g., asthma, psoriasis)).

As integrin family members play a role in immune response, Tango-71 nucleic acids, proteins, and modulators thereof can be used to treat immune related disorders, e.g., immunodeficiency disorders (e.g., HIV), viral disorders (e.g., infection by HSV), cell growth disorders, e.g., cancers (e.g., carcinoma, lymphoma, e.g., follicular lymphoma), autoimmune disorders (e.g., arthritis, graft rejection (e.g., allograft rejection), T cell autoimmune disorders (e.g., AIDS)), and inflammatory disorders (e.g., bacterial or viral infection, psoriasis, septicemia, cerebral malaria, inflammatory bowel disease (e.g., ulcerative colitis, Crohn's disease), arthritis (e.g., rheumatoid arthritis, osteoarthritis), allergic inflammatory disorders (e.g., asthma, psoriasis)).

As integrin family members play a role in cell growth, survival, proliferation, and migration, Tango-71 nucleic acids, proteins, and modulators thereof can be used to treat apoptotic disorders (e.g., rheumatoid arthritis, systemic lupus erythematosus, insulin-dependent diabetes mellitus) proliferative disorders (e.g., cancers, e.g., B cell cancers stimulated by TNF), and disorders abnormal vascularization (e.g., cancer). In addition,

71

Tango-71 nucleic acids, proteins, and modulators thereof can also be used to promote vascularization (angiogenesis).

As integrins are cell adhesion molecules, Tango-71 nucleic acids, proteins, and modulators thereof can be used to modulate disorders associated with adhesion and migration of cells, e.g., platelet aggregation disorders (e.g., Glanzmann's thromboasthemia, which is a bleeding disorders characterized by failure of platelet aggregation in response to cell stimuli), inflammatory disorders (e.g., leukocyte adhesion deficiency, which is a disorder associated with impaired migration of neutrophils to sites of extravascular inflammation), disorders associated with abnormal tissue migration during embryo development, and tumor metastasis.

1. Prophylactic Methods

In one aspect, the invention provides a method for preventing in a subject, a disease or condition associated with an aberrant expression or activity of a polypeptide of the invention, by administering to the subject an agent that modulates expression or at least one activity of the polypeptide. Subjects at risk for a disease that is caused or contributed to by aberrant expression or activity of a polypeptide of the invention can be identified by, for example, any or a combination of diagnostic or prognostic assays as described herein. Administration of a prophylactic agent can occur prior to the manifestation of symptoms characteristic of the aberrancy, such that a disease or disorder is prevented or, alternatively, delayed in its progression. Depending on the type of aberrancy, for example, an agonist or antagonist agent can be used for treating the subject. The prophylactic agents described herein, for example, can be used to treat a subject at risk of developing disorders such as disorders discussed for example, in Sections above relative to rhe uses of the sequences of the invention. For example, an antagonist of a Tango-71, Tango-79, or Tango-81 protein may be used to modulate or treat an immunological disorder. The appropriate agent can be determined based on screening assays described herein.

2. Therapeutic Methods

Another aspect of the invention pertains to methods of modulating expression or activity of a polypeptide of the invention for therapeutic purposes. The modulatory method of the invention involves contacting a cell with an agent that modulates one or more of the activities of

the polypeptide. An agent that modulates activity can be an agent as described herein, such as a nucleic acid or a protein, a naturally-occurring cognate ligand of the polypeptide, a peptide, a peptidomimetic, or other small molecule. In one embodiment, the agent stimulates one or more of the biological activities of the polypeptide. Examples of such stimulatory agents include the active polypeptide of the invention and a nucleic acid molecule encoding the polypeptide of the invention that has been introduced into the cell. In another embodiment, the agent inhibits one or more of the biological activities of the polypeptide of the invention. Examples of such inhibitory agents include antisense nucleic acid molecules and antibodies. These modulatory methods can be performed *in vitro* (*e.g.*, by culturing the cell with the agent) or, alternatively, *in vivo* (*e.g.*, by administering the agent to a subject). As such, the present invention provides methods of treating an individual afflicted with a disease or disorder characterized by aberrant expression or activity of a polypeptide of the invention. In one embodiment, the method involves administering an agent (*e.g.*, an agent identified by a screening assay described herein), or combination of agents that modulates (*e.g.*, upregulates or downregulates) expression or activity. In another embodiment, the method involves administering a polypeptide of the invention or a nucleic acid molecule of the invention as therapy to compensate for reduced or aberrant expression or activity of the polypeptide.

Stimulation of activity is desirable in situations in which activity or expression is abnormally low or downregulated and/or in which increased activity is likely to have a beneficial effect. Conversely, inhibition of activity is desirable in situations in which activity or expression is abnormally high or upregulated and/or in which decreased activity is likely to have a beneficial effect.

This invention is further illustrated by the following examples that should not be construed as limiting. The contents of all references, patents and published patent applications cited throughout this application are hereby incorporated by reference.

Effective Dose

Toxicity and therapeutic efficacy of the polypeptides of the invention and the compounds that modulate their expression or activity can be determined by standard pharmaceutical procedures, using either cells in culture or experimental animals to determine the LD_{50} (the dose lethal to 50% of the population) and the ED_{50} (the dose therapeutically effective in 50% of the

73

population). The dose ratio between toxic and therapeutic effects is the therapeutic index and it can be expressed as the ratio LD_{50}/ED_{50}. Polypeptides or other compounds that exhibit large therapeutic indices are preferred. While compounds that exhibit toxic side effects may be used, care should be taken to design a delivery system that targets such compounds to the site of affected tissue in order to minimize potential damage to uninfected cells and, thereby, reduce side effects.

The data obtained from the cell culture assays and animal studies can be used in formulating a range of dosage for use in humans. The dosage of such compounds lies preferably within a range of circulating concentrations that include the ED_{50} with little or no toxicity. The dosage may vary within this range depending upon the dosage form employed and the route of administration utilized. For any compound used in the method of the invention, the therapeutically effective dose can be estimated initially from cell culture assays. A dose may be formulated in animal models to achieve a circulating plasma concentration range that includes the IC_{50} (that is, the concentration of the test compound which achieves a half-maximal inhibition of symptoms) as determined in cell culture. Such information can be used to more accurately determine useful doses in humans. Levels in plasma may be measured, for example, by high performance liquid chromatography.

Formulations and Use

Pharmaceutical compositions for use in accordance with the present invention may be formulated in conventional manner using one or more physiologically acceptable carriers or excipients.

Thus, the compounds and their physiologically acceptable salts and solvates may be formulated for administration by inhalation or insufflation (either through the mouth or the nose) or oral, buccal, parenteral or rectal administration.

For oral administration, the pharmaceutical compositions may take the form of, for example, tablets or capsules prepared by conventional means with pharmaceutically acceptable excipients such as binding agents (for example, pregelatinised maize starch, polyvinylpyrrolidone or hydroxypropyl methylcellulose); fillers (for example, lactose, microcrystalline cellulose or calcium hydrogen phosphate); lubricants (for example, magnesium stearate, talc or silica); disintegrants (for example, potato starch or sodium starch glycolate); or

74

wetting agents (for example, sodium lauryl sulphate). The tablets may be coated by methods well known in the art. Liquid preparations for oral administration may take the form of, for example, solutions, syrups or suspensions, or they may be presented as a dry product for constitution with water or other suitable vehicle before use. Such liquid preparations may be prepared by conventional means with pharmaceutically acceptable additives such as suspending agents (for example, sorbitol syrup, cellulose derivatives or hydrogenated edible fats); emulsifying agents (for example, lecithin or acacia); non-aqueous vehicles (for example, almond oil, oily esters, ethyl alcohol or fractionated vegetable oils); and preservatives (for example, methyl or propyl-p-hydroxybenzoates or sorbic acid). The preparations may also contain buffer salts, flavoring, coloring and sweetening agents as appropriate. Preparations for oral administration may be suitably formulated to give controlled release of the active compound.

For buccal administration the compositions may take the form of tablets or lozenges formulated in conventional manner.

For administration by inhalation, the compounds for use according to the present invention are conveniently delivered in the form of an aerosol spray presentation from pressurized packs or a nebulizer, with the use of a suitable propellant, for example, dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane, carbon dioxide or other suitable gas. In the case of a pressurized aerosol the dosage unit may be determined by providing a valve to deliver a metered amount. Capsules and cartridges of, for example, gelatin for use in an inhaler or insufflator may be formulated containing a powder mix of the compound and a suitable powder base such as lactose or starch.

The compounds may be formulated for parenteral administration by injection, for example, by bolus injection or continuous infusion. Formulations for injection may be presented in unit dosage form, for example, in ampoules or in multi-dose containers, with an added preservative. The compositions may take such forms as suspensions, solutions or emulsions in oily or aqueous vehicles, and may contain formulatory agents such as suspending, stabilizing and/or dispersing agents. Alternatively, the active ingredient may be in powder form for constitution with a suitable vehicle, for example, sterile pyrogen-free water, before use.

The compounds may also be formulated in rectal compositions such as suppositories or retention enemas, for example, containing conventional suppository bases such as cocoa butter or other glycerides.

1855,2058-001

In addition to the formulations described previously, the compounds may also be formulated as a depot preparation. Such long acting formulations may be administered by implantation (for example subcutaneously or intramuscularly) or by intramuscular injection. Thus, for example, the compounds may be formulated with suitable polymeric or hydrophobic materials (for example as an emulsion in an acceptable oil) or ion exchange resins, or as sparingly soluble derivatives, for example, as a sparingly soluble salt.

The compositions may, if desired, be presented in a pack or dispenser device that may contain one or more unit dosage forms containing the active ingredient. The pack may for example comprise metal or plastic foil, such as a blister pack. The pack or dispenser device may be accompanied by instructions for administration.

The therapeutic compositions of the invention can also contain a carrier or excipient, many of which are known to skilled artisans. Excipients that can be used include buffers (for example, citrate buffer, phosphate buffer, acetate buffer, and bicarbonate buffer), amino acids, urea, alcohols, ascorbic acid, phospholipids, proteins (for example, serum albumin), EDTA, sodium chloride, liposomes, mannitol, sorbitol, and glycerol. The nucleic acids, polypeptides, antibodies, or modulatory compounds of the invention can be administered by any standard route of administration. For example, administration can be parenteral, intravenous, subcutaneous, intramuscular, intracranial, intraorbital, opthalmic, intraventricular, intracapsular, intraspinal, intracisternal, intraperitoneal, transmucosal, or oral. The modulatory compound can be formulated in various ways, according to the corresponding route of administration. For example, liquid solutions can be made for ingestion or injection; gels or powders can be made for ingestion, inhalation, or topical application. Methods for making such formulations are well known and can be found in, for example, "Remington's Pharmaceutical Sciences." It is expected that the preferred route of administration will be intravenous.

It is recognized that the pharmaceutical compositions and methods described herein can be used independently or in combination with one another. That is, subjects can be administered one or more of the pharmaceutical compositions, e.g., pharmaceutical compositions comprising a nucleic acid molecule or protein of the invention or a modulator thereof, subjected to one or more of the therapeutic methods described herein, or both, in temporally overlapping or non-overlapping regimens. When therapies overlap temporally, the therapies may generally occur in any order and can be simultaneous (e.g., administered simultaneously together in a composite

composition or simultaneously but as separate compositions) or interspersed. By way of example, a subject afflicted with a disorder described herein can be simultaneously or sequentially administered both a cytotoxic agent which selectively kills aberrant cells and an antibody (e.g., an antibody of the invention) which can, in one embodiment, be conjugated or linked with a therapeutic agent, a cytotoxic agent, an imaging agent, or the like.

Equivalents

Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. Such equivalents are intended to be encompassed by the following claims.